VOLUME 38 FEBRUARY 1960 NUMBER 1

Canadian Journal of Zoology

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

Canadian Journal of Zoology

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VOLUME 38 1960

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STUDIES ON THE DEVELOPMENT OF THE KIDNEY OF THE PACIFIC PINK SALMON (ONCORHYNCHUS GORBUSCHA (WALBAUM))

III. THE DEVELOPMENT OF THE MESONEPHROS WITH PARTICULAR REFERENCE TO THE MESONEPHRIC TUBULE[1]

J. D. NEWSTEAD[2] AND PETER FORD

Abstract

The origin and subsequent elaboration of the mesonephric tubules from condensation of cells derived from the intermediate cell mass is described from the earliest appearance of condensations to the differentiated stage in the year-old fingerling. It is noted that both the pronephric and mesonephric kidneys are present together for a considerable period of the early life of the pink salmon, though it is unlikely that both function simultaneously.

Introduction

The teleost kidney is derived in each segment from the intermediate cell mass, which separates from the somite early in development, and somewhat later from the lateral plate mesoderm. The intermediate cell mass becomes differentiated posterior to the pronephros into three elements:

1. The vascular strand, from which develop the aorta, cardinal veins (or vein, in those species in which only one postcardinal develops), mesonephric glomeruli, and myeloid tissue of the kidney, as well as other tissues not directly related to the kidney (6). Formation of the vascular strand is supposed to occur by migration of cells from the medial aspect of the intermediate cell mass to the mid-line, where they form a dense mass (10).

2. The segmental duct rudiment. There is no direct evidence for the supposed segmental origin of the duct rudiment in teleosts, but several descriptive studies have suggested such to be the case (13, 14). We have found some indication in our own material that the segmental duct in *Oncorhynchus gorbuscha* may originate *in situ* rather than (5) by growth posteriad of the pronephric tubules.

[1]Manuscript received August 26, 1959.
Contribution from the Department of Zoology, University of British Columbia, Vancouver, British Columbia. This study was supported by a grant in aid of research from the National Research Council of Canada.
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[The previous number of Can. J. Zoology (37, 817–1130) was issued February 8, 1960.]

3. The mesonephrogenic material proper, from which develop the mesonephric tubules and collecting ducts. The name "mesonephrogenic bridge" proposed by Moghe (11) for this material, which extends in the form of a bridge from one duct to the other between the aorta and cardinal vein, is retained in this account as being more descriptive than some of the other proposed nomenclature (mesonephric blastema (8), mesonephric mesenchyme (10)). Although the bridge is said to differentiate along the entire trunk region from the posterior limit of the pronephros to the posterior limit of the kidney rudiment, usually no nephric structures develop opposite the somites immediately posterior to the pronephros or at the extreme caudal end of the mesonephric kidney (7).

The concern of the present report is primarly with the development and structure of the mesonephric tubules derived from this mesonephrogenic bridge.

Materials and Methods

Collection and treatment of material has been described in detail elsewhere (5).

Developmental History

The intermediate cell mass in the pink salmon becomes separated from the somite in each segment shortly after the appearance of the somites; the attachment to the lateral plate is retained, however, for some time (Fig. 1). No segmentation of the intermediate cell mass is apparent at any time in the mesonephric region, nor do the mesonephric tubules show any tendency toward segmental arrangement during the early stages. The duct on either side separates as a solid rod of cells continuous anteriorly with the pronephric fold. Tubulation of the ducts follows their segregation almost immediately. Movement of cells from the medial aspect of the intermediate cell mass on either side establishes the vascular strand as a medially located accumulation of basophilic cells (Fig. 2). Differentiation of the dorsal aorta and right cardinal vein commences in the anterior region of the mass and proceeds posteriorly. The left cardinal vein appears as a discrete vessel only in the pronephric region.

FIG. 1. Transverse section of 3.45-mm embryo through 16th segment to show triangular intermediate cell mass. ×125

FIG. 2. Transverse section of 4.4-mm embryo through 20th somite to show separation of duct rudiment and vascular strand. ×125.

FIG. 3. Transverse section of 4.4-mm embryo through 10th somite to show the early stage of the mesonephros, the aorta, cardinal vein, mesonephrogenic cord above the gut, and mesonephric ducts. ×200

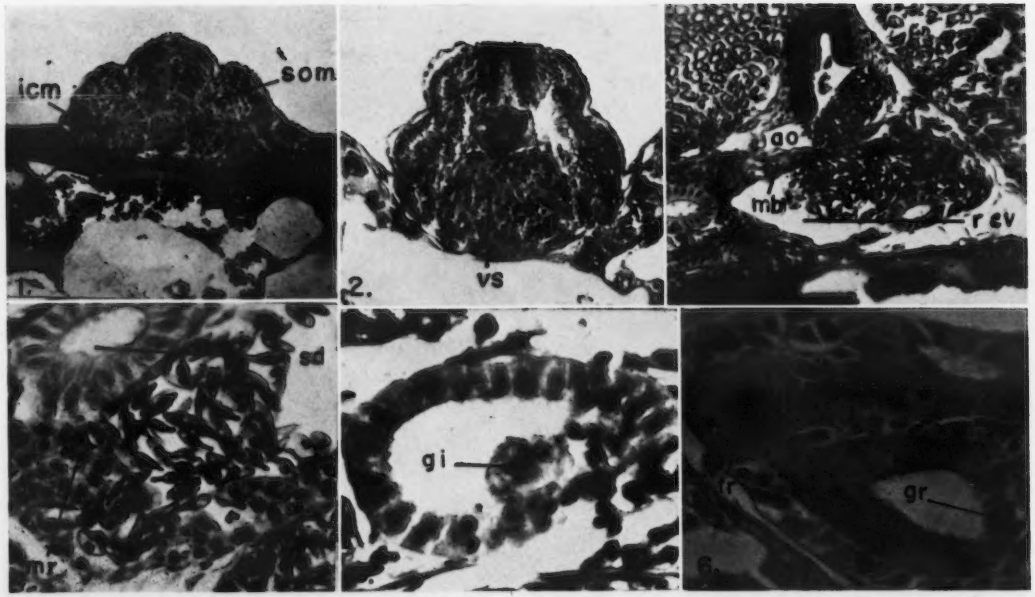
FIG. 4. Transverse section of left side of kidney of 16.66-mm embryo to show mesonephric condensation. ×450

FIG. 5. Transverse section of kidney of 19.8-mm alevin to show incipient glomerular invagination. ×450

FIG. 6. Section of Bowman's capsule and avascular glomerular invagination and tubule rudiment of 19.8-mm alevin. ×450

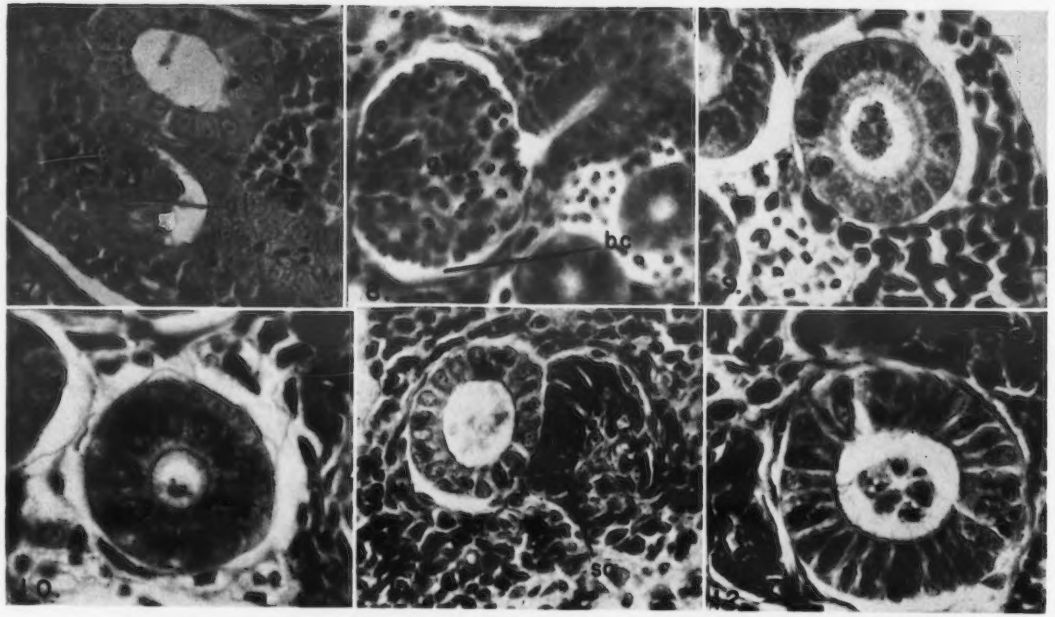
ABBREVIATIONS: *ao*, aorta; *bc*, Bowman's capsule; *gi*, glomerular ingrowth; *gr*, glomerular rudiment; *gl*, glomerulus; *icm*, intermediate cell mass; *mb*, mesodermal bridge; *mr*, mesonephrogenic rudiment; *rcv*, right cardinal vein; *sc*, secondary condensation; *sd*, segmental duct; *som*, somite; *vs*, vascular strand.

PLATE I



Newstead and Ford—Can. J. Zool.

PLATE II



Newstead and Ford—Can. J. Zool.

The dorsomedially located cells (mesonephrogenic bridge rudiment) of the intermediate cell mass remain in close association with the ventral aspect of the somite and the dorsal aspect of the segmental duct for some time; when differentiation of the cardinal vein and aorta has occurred, the cells of the bridge are moved from their earlier position to form a narrow cellular bridge passing from one duct to the other between the two blood vessels (Fig. 3). The bridge appears to be longitudinally continuous throughout the entire length of the trunk posterior to the pronephros (from the 12th to the 39th segments), though it is not always complete in any one section. Primary mesonephric rudiments appear as condensations of the cells of the mesonephrogenic bridge where it is closely applied to the dorsal surfaces of the ducts (Figs. 3, 4). The earliest condensations do not show a segmental arrangement, although they are invariably located toward the posterior end of the mesonephros. Subsequent primary tubules are added for the most part anterior to those first formed. Shortly after their initial appearance the rudiments commence to elongate until they form cylinders curved around the ducts. The proximal ends of the cylinders penetrate the duct wall, but the tubules do not form and open to the ducts until after the formation of the Bowman capsule (Fig. 5). Rapid proliferation of cells at the distal end of each rudiment results in the formation of a bulbous swelling which increases in size and soon hollows out to form a Bowman capsule (Fig. 5). Distention of the capsule extends the originally columnar epithelium into a squamous form. The capsular lumen extends along the tubule and finally breaks through into the segmental duct. At a point approximately opposite the tubule opening, the Bowman capsule wall is invaginated by a solid mass of cells (Fig. 6) whose origin seems to be from the vascular strand. The appearance of glomerular capillaries in the mass (Figs. 7, 8) is followed by connection of the newly formed glomerular tuft to the aorta by a glomerular arteriole.

Following the appearance of the glomerulus the tubular epithelium, which until this time was of low simple columnar cells, becomes differentiated into five regions:

1. A glomerular neck segment of laterally compressed, tall columnar cells (Fig. 8).

2. A segment characterized by its low columnar epithelium and relatively high brush border. It is this segment to which Edwards (2) gives the name "first major segment." This segment is the first of the so-called major segments to differentiate (Fig. 9).

FIG. 7. Transverse section of kidney of 26.9-mm frog to show start of vascularization of the glomerulus. ×450

FIG. 8. Glomerulus and capsule of fully differentiated nephron and tubule neck segment. ×350

FIG. 9. Transverse section of first major segment. ×350

FIG. 10. Transverse section of second major segment with tubule showing reduced lumen and lower border. ×450

FIG. 11. Transverse section of third major (distal) segment and secondary condensation. ×400

FIG. 12. Transverse section of collecting duct. ×350

3. A segment of taller columnar cells with a lower brush border, designated "second major segment" (2). This segment is characterized by extreme basophilia of the cytoplasm and by the much narrowed tubular lumen (Fig. 10).

4. A segment composed of cuboidal cells, and having a relatively wide tubular lumen (third major segment of Edwards (Fig. 11)). When secondary condensations commence to appear they are located close to the junction of this segment with the collecting segment.

5. A collecting segment of tall columnar epithelium, differentiated from the segmental duct in cross-section only by the greater diameter of the latter (compare Figs. 12, 13). This collecting segment becomes the common collecting duct after secondary tubules are added to the primary nephron.

Mesonephric condensations are first found in embryos about 15 mm long, in which the pronephros has achieved its maximum development. Thereafter, there is a rapid increase in the number of rudiments, most of the newly formed condensations being anterior to those first laid down. By about 19 mm, when the first incipient Bowman capsules are appearing, there are an average of about 50 rudiments present in different stages of elaboration. Most show a degree of elongation and swelling of the distal end of the tubule; however, recent condensations are also detectable especially in the anterior region. At this time the only arterial blood reaching the kidney is that brought directly to the corpuscles of Stannius (5), which show the first signs of vascularization at about this time. Venous blood is brought to the kidney by way of paired dorsal and ventral intersegmental veins which diversify into the capillary network permeating the myeloid tissue. Venous drainage is achieved by way of the single median postcardinal vein which develops as the right postcardinal (Fig. 3).

Glomerular invaginations appear very shortly after the cavitation of the Bowman capsule. Their appearance is almost synchronous with the achievement of the squamous form of the capsular epithelium (about 20 mm) (Fig. 6), and again the more posterior rudiments are the first affected. Commencement of vascularization is delayed until considerably later (27 mm) by which time the glomerular rudiment almost entirely fills the Bowman capsule (Fig. 7). Well-formed glomeruli (Fig. 14) appear only at about the 29-mm stage, when secondary Bowman capsules are also found (Fig. 15). Soon after, the first signs of pronephric involution are seen in the gradual occlusion of the pronephric arteriole and increasing fibrosis of the glomerulus itself. Arterial blood is supplied to the mesonephric glomeruli from paired intersegmental arteries which enter the dorsolateral apices of the trapezoid kidney. Apparently some of the paired arteries later degenerate since Udvardy reports (personal communication) that in the mature pink salmon the arrangement of the arteries is irregular. The change in kidney form from the semilunar shape seen in the young alevins to the trapezoid shape typical of the fry is apparently due to proliferation of myeloid tissue and tubules which become very abundant (Fig. 16). Further development comprises the addition of

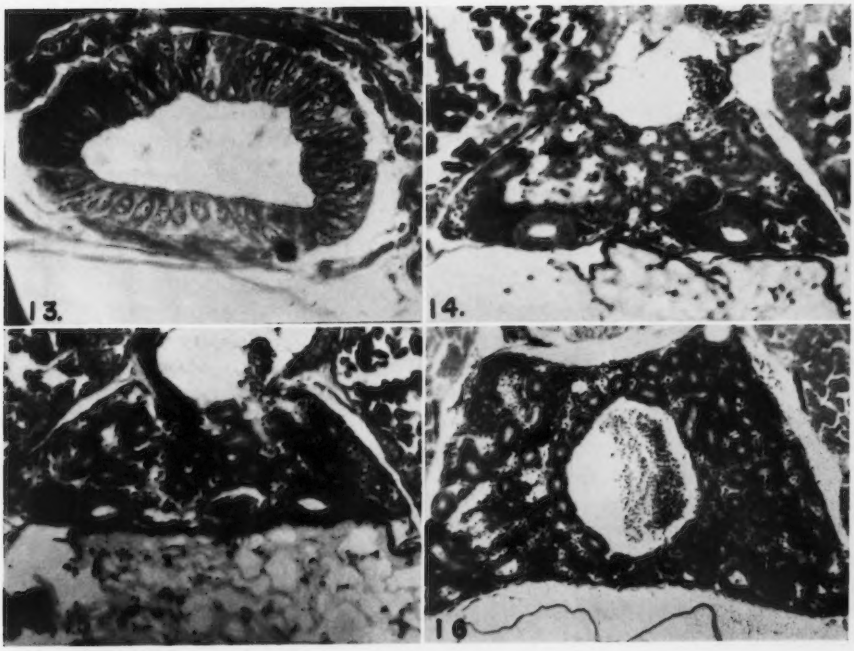


FIG. 13. Transverse section of segmental duct. ×325
FIG. 14. Transverse section of kidney of 29.10-mm frog to show well-formed glomerulus. ×125
FIG. 15. Transverse section of kidney of 29.78-mm frog. ×125
FIG. 16. Typical transverse section of kidney of 64-mm fingerling in midtrunk region showing central cardinal vein, pronounced tubulation, myeloid tissue, arteriole entering upper right corner, venule entering lower left. ×125

more primary tubules opening directly to the segmental ducts, and of further secondary and later generations of tubules opening into the primary tubules. Although signs of pronephric degeneration appear rather early in the course of mesonephric development, and the pronephros apparently is rendered non-functional at an early stage of degeneration by closure of the nephrostomes (5), pronephric glomeruli and tubules were still identifiable in the oldest specimens (120 mm, 1 year old) examined by us.

Discussion

The prolonged attachment of the teleost intermediate cell mass to the lateral plate has been the cause of considerable confusion in the interpretation of the early stages of kidney development. The origin of the mesonephric condensations (called "blastema") from the intermediate cell mass was established for the teleost kidney (10). Hoffman had described the appearance of teleost tubules from condensations of cells near the segmental ducts, but was unable to locate the origin of the cells themselves (9). Rosenberg (12) believed that the rudiments arose by condensation of "parietal" cells derived from the wall of the dorsal aorta. Felix described the appearance of primary tubules as outgrowths of the walls of the segmental ducts, and of secondary permanent tubules as condensations of unknown origin (4). Felix's primary rudiments were, however, probably the rudimentary corpuscles of Stannius (1). Emery correctly described the origin of mesonephric rudiments as condensations of cells in a bridge extending from one duct to the other between the cardinal vein and the aorta, but suspected that the bridge cells took their origin from the peritoneal epithelium (3). Moghe described a similar bridge of cells in which mesonephric condensations appeared (11) and was able to verify the finding of Maschkowzeff (10) that the bridge itself was derived from the intermediate cell mass, as has also proved to be the case in the present study. The reported non-segmental nature of the intermediate cell mass in the mesonephric region and the non-metameric arrangement of the early mesonephric rudiments is in accordance with the views of most investigators of teleost kidney development (6).

Fraser, in her cited review of literature pertinent to the development of kidneys in all classes of vertebrates (6), reports that mesonephric Bowman capsules develop as dilations of the free end of already tubulated rudiments. Moghe (11) has reported that in *Thynnichthys sandkohl*, the mesonephric Bowman capsules develop as independent spherical condensations of the bridge cells; the rudiments become hollowed out and subsequently connect to the mesonephric tubule rudiment, already patent. The course of capsule development in our species is different from either of these previously reported modes; the capsule develops by proliferation of the cells at the free end of the solid tubule rudiment, and subsequent cavitation of the bulb so formed, the lumen of which then extends into the tubule. The vascularization of glomeruli in birds prior to their connection with the aorta has been reported (Davies and Davies (in 6)) and in fish in the pronephros of *Oncorhynchus gorbuscha* (5).

The formation of mesonephric glomeruli is apparently similar to that of the pronephric glomerulus in this respect. The initial formation of the glomerulus as an avascular mass of invaginated cells is of interst in itself. A somewhat similar mode of glomerular formation has been reported by Moghe (11).

Summary

1. The intermediate cell mass in the mesonephric region differentiates into three distinct elements:

(*a*) the vascular strand, which gives rise primarily to vascular tissue and myeloid tissue;

(*b*) the segmental duct rudiment;

(*c*) the mesonephrogenic bridge rudiment, from which develop the mesonephric tubules.

2. The mesonephric tubules develop from condensations of the mesonephrogenic bridge cells. The condensations elongate and develop a hollow Bowman capsule at their distal end. The lumen of the capsule subsequently extends into the tubule, and finally enters the segmental duct.

3. The mesonephric glomeruli originate as solid invaginations of the capsular wall, later become vascularized, and connect to the aorta only after primary vascularization has occurred.

4. Although the pronephros and mesonephros coexist for a considerable period, it is suspected that for most of this period the pronephros is probably not functional.

Acknowledgments

Thanks are due Dr. W. S. Hoar of this department and the director and staff of the Pacific Biological station at Nanaimo, all of whom assisted with the collection of specimens from marine environments. We are indebted to Miss Martha Nagai, Mr. Bernard Cox, Miss Janet Goodman, and Miss Sachiko Tabata for histological preparation.

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OBSERVATIONS ON SOME INSECT PREDATORS OF BLACK FLIES (DIPTERA:SIMULIIDAE) OF ALGONQUIN PARK, ONTARIO[1]

B. V. PETERSON[2] AND D. M. DAVIES[3]

Abstract

Insect predators of black flies are common although their predatory acts are infrequently observed. Observations are presented on black-fly predation by various species of the dipterous families Empididae, Dolichopodidae, Ephydridae, and Tendipedidae. A case of cannibalism among larvae of *Simulium venustum* is reported. Other insect predators of black flies mentioned include various species of Trichoptera, especially species of the genus *Hydropsyche*; Hymenoptera; and Odonata. Non-insect predators of simuliids that are mentioned include spiders, and a leech, *Haemopis marmorata* (Say). In Algonquin Park, Ontario, larvae of species of the trichopteran genus *Hydropsyche* appeared to be the most important predators of simuliid larvae, while dance flies were the most important predators of adult black flies.

Introduction

Numerous instances of insects attacking and feeding on the adult and immature stages of black flies have come to the attention of the authors. Brief references to the natural enemies of Simuliidae are scattered in the literature, but collected information is contained only in the papers of Bequaert (2), Twinn (24), Grenier (13, 14), Vargas (27), and Crisp (5).

This paper summarizes the observations on insect predators of black flies made in Algonquin Park, Ontario, during the spring and summer of 1955. A number of these observations were made in or near the sluiceway of the Lake Sasajewun dam. The cement sluiceway measured 35 ft long, 12 ft wide, and 10 ft high (inside). Four or five wooden drop-logs at the lake side of the sluiceway held back a 3–4 ft depth of water. Early in the season, and intermittently thereafter, a thin sheet of water flowed over at least part of the top of the wooden drop-logs. In the sluiceway, and on its walls, as well as on the wooden drop-logs, a number of insect predators found a bountiful black-fly "hunting ground".

Diptera

Empididae[4]

Published reports on empidid predation of black flies are few (1, 25, 26, 28); however, the authors observed dance-fly predation on several species of

[1]Manuscript received July 27, 1959.
Contribution from the Entomology Laboratory, Research Branch, Canada Department of Agriculture, Guelph, Ontario, and the Department of Biology, Hamilton College, McMaster University, Hamilton, Ontario. Supported by a grant from the National Research Council of Canada.
[2]Entomology Laboratory, P.O. Box 248, Guelph.
[3]Department of Biology, Hamilton College.
[4]NOTE ADDED IN PROOF: After this paper was in proof, the empidid flies referred to in the text and in Table I as *Roederiodes* sp. nr. *juncta* were identified as *R. juncta* Coquillet by Dr. J. G. Chillcott.

black flies in Algonquin Park during the summer of 1955. The most commonly collected empidid predators of black flies were unnamed species of the genera *Rhamphomyia* (subgenera *Pararhamphomyia* and *Megacyttarus*), *Hilara*, and *Roederiodes*. Less common dance-fly predators included *Platypalpus xanthopodus* Melander, *Syndyas dorsalis* Loew, *Oedalia ohioensis* Melander, and *Hemerodromia melanosoma* Melander.

Many instances of empidid predation occurred while female black flies were in oviposition swarms, and in combined mating and oviposition swarms. Such predation occurred most frequently in the evenings but also was observed to occur in the early morning hours of warm days, and in the afternoon in sheltered and shaded places such as in the sluiceway of the Lake Sasajewun dam. Only once was predation observed in a simuliid mating swarm away from water. This occurred on June 3, at 7.00 p.m., when males and females of *Simulium venustum* Say formed a mating swarm about 7 ft above the ground, in an open stand of white pine and white spruce, about 500 yd from the South Madawaska River. One female of *Rhamphomyia* (*P.*) *basalis* Loew and one male of *R.* (*P.*) *clauda* Coquillett were netted with their prey from the swarm.

At 10.00 a.m. on May 24, at the outlet to Davies Bog, low- and slow-flying females of *Roederiodes* sp. (nr. *juncta*) were observed to fly up and down the stream waiting for newly emerged black-fly adults (and possibly other insects) to escape from the water. As the vulnerable black-fly adults left the water, they were pounced upon immediately by the empidid females and carried to the stream-side vegetation where they were consumed. Similar predation by empidids on mosquitoes was reported by Frohne (11, 12).

On two other occasions a similar type of skimming predation was noted to occur over Lake Sasajewun about 300 ft out from shore. On June 7, at 7.20 p.m., while we were sweeping from a canoe for simuliids flying close to the surface of the lake, we netted a male of *Hilara* sp. that had clutched in its legs a female of *Eusimulium euryadminiculum* Davies (? = *E. canonicolum* Dyar and Shannon). A male of *Rhamphomyia* (*P.*) sp. 'O' was collected under similar circumstances on the evening of June 11.

Empidids were noted to capture resting adult black flies. On the afternoon of June 30, a number of *Simulium decorum* Walker females were found resting on the walls of the sluiceway, 6–8 in. above the surface of the stream. These females apparently had recently oviposited because some specimens collected contained only a few mature eggs. These flies were not easily disturbed either by the writers, or by the *Hilara* sp. females that were crawling among, and selectively feeding on them. A similar situation occurred on July 17, at 1.00 p.m., when unresisting females of *Simulium decorum* were under constant attack by females of *Hemerodromia melanosoma*.

On July 13, at 4.00 p.m., large numbers of males and females of *Roederiodes* sp. (nr. *juncta*) were seen crawling among a group of *Simulium decorum* larvae and pupae. These immature forms were on the east wall of the sluiceway, a little above the surface of the water, but were kept moist by the surging action of the stream. A few of the empidids were noted attacking dead,

deteriorating, and sometimes moldy pupae but none were observed to attack living pupae or the larvae. Wirth (28) observed adults of the empidid genus *Wiedemannia* dragging simuliid larvae from the water's edge in swift streams of the Sierra Nevada Mountains in California. Vaillant (25), in 1951, described *W.* (*R.*) *ouedorum*, and in 1953 (26), described *Hemerodromia* (*M.*) *seguyi*, the larvae of which are predaceous on black-fly larvae. Vaillant (26) stated that the empidid larva, when feeding, buries its head in a black-fly larva and sucks up the body contents.

On June 13, at 6.50 p.m., a female of *Rhamphomyia* (*P.*) *basalis* was collected from a spider web, still holding a female of *Simulium decorum* in her legs.

On none of the above occasions was dance-fly predation seen to be coupled with their mating activity. Both male and female empidids were observed to prey on black flies, an observation apparently in contrast to those of Frohne (11) on *Rhamphomyia*, in which only the males were active predators on mosquitoes.

Table I summarizes the observations on empidid predators of black flies in Algonquin Park.

Dolichopodidae

Adult dolichopodids were observed to prey on adult black flies only three times. The first occurred on June 15, at 4.00 p.m., when a female of *Chrysotus* sp. was netted at the Tea Lake dam. This dolichopodid held a *Simulium venustum* female which was parasitized by a mermithid nematode.

A male of *Chrysotus obliquus* Loew, clutching a female of *Simulium venustum*, was taken on June 16, over rapids of the South Madawaska River, just south of the airport. Another female of *S. venustum* was removed from the grasp of a male *Rhaphium effilatum* Wheeler, netted flying over rapids of the North Madawaska River, below Lake Sasajewun, on July 7.

Twinn (24) gave an account of *Dolichopus splendidulus* Loew and *Chrysotus* sp. that attacked black-fly larvae which were exposed above the water surface due to fluctuations of the water level.

Ephydridae

A single female of *Ochthera mantis* (Degeer) was collected in the sluiceway on May 23, from a swarm containing gravid females of *Simulium decorum* and *Prosimulium fuscum* Syme and Davies, and non-gravid females of *Simulium venustum*. Although the ephydrid female was not observed to take any black-fly prey, it was probably on the hunt, since its abdomen was not distended with food, and both the adults and larvae of *Ochthera* are known to be predaceous on smaller insects (28).

Tendipedidae

Samples of material containing eggs and first instar larvae of *Simulium decorum* and *S. venustum*, as well as several tendipedid larvae, were collected on June 20, from the wooden drop-logs of the Lake Sasajewun dam, and taken to the laboratory for study. While the hatching process of black-fly

TABLE I

Summary of observed dance-fly predation on black flies in Algonquin Park, Ontario, during the summer of 1955

Predators			Prey			Time (E.S.T.)	Date	Locality
Species	Sex	Activity*	Species	Sex	Activity†			
Roederiodes sp. nr. *juncta*	♂, ♀♀	SP	*Simulium venustum*	♀♀	F	7.30 p.m.	May 21	Sluiceway, Lake Sasajewun dam
Roederiodes sp. nr. *juncta*	♀	EP	*Simulium venustum*	♀	EF	10.00 a.m.	May 24	Davies Bog
Rhamphomyia basalis	♀	SP	*Simulium venustum*	♀	M	7.00 p.m.	June 3	Airport
Rhamphomyia clauda	♂	SP	*Simulium venustum*	♀	M	7.00 p.m.	June 3	Airport
Rhamphomyia sp.	♀	SP	*Simulium venustum*	♀	F	11.00 a.m.	June 6	Mile 1.5, Tote road, E. of Lake Sasajewun
Rhamphomyia clauda	♂	SP	*Simulium venustum*	♀	F	2.00 p.m.	June 6	Along banks of S. Madawaska River, S. of airport
Hilara sp.	♂	SK	*Eusimulium euryadminiculum*	♀	F	7.20 p.m.	June 7	300 ft out from shore, over Lake Sasajewun
Rhamphomyia sp. O	♂	SK	*Eusimulium euryadminiculum*	♀	F	7.20 p.m.	June 11	300 ft out from shore, over Lake Sasajewun
Rhamphomyia basalis	♀		*Simulium decorum*	♀		6.50 p.m.	June 13	Trapped in spider web at the Lake Sasajewun dam
Hilara sp.	♂	SK	*Simulium rugglesi*	♀	F	3.00 p.m.	June 15	Marsh's Falls, Oxtongue River, 30 miles S.E. of Algonquin Park
Rhamphomyia sp. B	♂, ♀♀	SP	*Simulium venustum*	♀♀	F	2.00 p.m.	June 16	Along banks of S. Madawaska River, S. of Airport
Syndyas dorsalis	♀	SP	*Simulium venustum*	♀	F	7.38 p.m.	June 19	Near duck pens on S.W. shore of Lake Sasajewun
Rhamphomyia sp. B	♂	SP	*Simulium decorum*	♀	O	8.15 a.m.	June 27	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Hilara sp.	♀	SP	*Simulium decorum*	♀	O	8.15 a.m.	June 27	Rapids, N. Madawaska river, ¼ mile S. of Lake Sasajewun
Roederiodes sp. nr. *juncta*	♂	SP	*Simulium decorum*	♀	O	2.30 p.m.	June 30	Sluiceway, Lake Sasajewun dam
Hilara sp.	♀	LP	*Simulium decorum*	♀	R	2.30 p.m.	June 30	Walls of sluiceway, Lake Sasajewun dam
Oedalia ohioensis	♀	SP	*Simulium venustum*	♀	F	3.00 p.m.	June 30	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Platypalpus xanthopodus	♀	SP	*Simulium venustum*	♀	F	3.00 p.m.	June 30	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Roederiodes sp. nr. *juncta*	♂, ♀♀	SP	*Simulium decorum*	♂, ♀♀	MO	3.50 p.m.	July 13	Sluiceway, Lake Sasajewun dam
Roederiodes sp. nr. *juncta*	♂♂, ♀♀	LP	*Simulium decorum*	Dead pupae		4.00 p.m.	July 13	Walls of sluiceway, Lake Sasajewun dam
Roederiodes sp. nr. *juncta*	♂, ♀♀	SP	*Simulium decorum*	♀♀	O	8.00 a.m.	July 14	Sluiceway, Lake Sasajewun dam
Roederiodes sp. nr. *juncta*	♂♂	SP	*Simulium venustum*	♀♀	O	9.00 a.m.	July 16	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Hemerodromia melanosoma	♀	LP	*Simulium decorum*	♀	R	1.00 p.m.	July 17	Walls of sluiceway, Lake Sasajewun dam
Roederiodes sp. nr. *juncta*	♂	SP	*Simulium decorum*	♀	O	7.00 p.m.	July 17	Sluiceway, Lake Sasajewun dam
Hilara sp.	♀	SP	*Simulium decorum*	♀	O	7.20 p.m.	Aug. 6	Sluiceway, Lake Sasajewun dam

*Predator activity: SP—predation while flying in black-fly swarms; LP—predation while landed on some support; EP—skimming over water surface to prey on emerging adults; SK—general skimming over water surface.

†Prey activity: F— general flight; EF—emergence flight; M—mating flight; O—oviposition flight; MO—combination mating and oviposition flight; R—resting.

eggs was being observed, a tendipedid larva was seen to ingest a complete, newly hatched black-fly larva. The anterior one-third of the tendipedid larva projected from its tubular, gelatinous shelter, and by means of vigorous undulations and back and forth motions of its body, it created a current of water that drew additional black-fly larvae and eggs near its shelter. The tendipedid larva plunged at its prey but had difficulty catching and holding the small, wriggling, black-fly larvae. Many unsuccessful attempts were made and the larva groped about, seizing empty shells of black-fly eggs before a second simuliid larva was finally caught and consumed.

Wu (30) observed *Chironomus* (= *Tendipes*) larvae attacking living black-fly larvae and pupae in an aquarium. Several authors have observed and reported that tendipedid larvae were frequently found living inside the cocoons of several species of black flies, but the exact nature of the relations between these larvae and simuliid pupae remains unknown (2, 5, 8, 9, 13, 14, 21, 22).

Simuliidae

Many different kinds of insects are known to have cannibalistic tendencies, particularly under certain unfavorable conditions. Simuliid larvae apparently are no exception, at least those of *Simulium venustum*. Larvae of this species, collected on May 27 from the North Madawaska River above Lake Sasajewun, were observed alive in the laboratory. A mature larva grasped, and literally swallowed whole, several first instar larvae. With its mandibles, the mature larva grasped one of the much smaller larvae and forced it headfirst into the oral cavity. With a few subsequent pushes by the mandibles, the small larva was completely ingested. This behavior occurred in standing water in a stender dish and may not constitute a normal behavior in the natural environment. However, since simuliid larvae have been reported to feed on tendipedid larvae (23) and other animal organisms, it seems probable that such cannibalistic habits could occur in nature. Wu (30) observed that a few black-fly larvae fed on the deteriorating bodies of dead larvae in an aquarium.

Trichoptera[5]

The predaceous larvae of caddis flies sometimes were found closely associated with the immature stages of black flies. More often, however, few, if any, black-fly larvae were found in close proximity to concentrations of caddis fly larvae. Species of the genus *Hydropsyche* were the most common and numerous trichopteran predators of black-fly larvae, while species of other genera were only occasionally observed to feed on them. All the observed cases of trichopteran predation on black-fly larvae, reported below, were verified by analyses of the gut contents of the trichopteran larvae.

[5]Unfortunately, the present status of knowledge regarding larvae of most caddis-fly genera such as *Hydropsyche*, *Cheumatopsyche*, *Chimarra*, etc. does not permit specific identifications to be made. Adults of these genera which were collected include: *Cheumatopsyche analis* (Banks) (Hydropsychidae), *Chimarra aterrima* Hagen, and *Chimarra obscura* (Walker) (Philopotamidae). In addition, adults of the following species were collected: *Lype diversa* (Banks) and *Phylocentropus placidus* (Banks) (Psychomyiidae).

On July 7, a small colony of *Hydropsyche* sp. larvae was found on the upstream portion of a large rock, and a larger number of small black-fly larvae occurred a few inches downstream on the same rock. This rock formed part of a sloping 15- to 20-ft waterfall in the South Madawaska River, and most of the surrounding rocks were covered, rather abundantly, by groups of larvae of *Simulium venustum* and *S. vittatum* Zetterstedt. Observations over a period of several days showed a progressive downstream migration of the caddis fly colony and a corresponding decrease in the number of black-fly larvae on the rock. *Hydropsyche* larvae were observed on several different occasions, feeding on the smaller simuliid larvae. Whether the actual decrease of the number of black-fly larvae was due largely to the caddis-fly predation, or to the migration of the black-fly larvae, is not known. However, the former seemed to be the case because of the predation observed, and because the groups of black-fly larvae on the surrounding rocks showed no obvious migratory trends.

On July 17, numerous larvae of *Cheumatopsyche* sp. and *Chimarra* sp. were observed feeding on the eggs and larvae, and occasionally on the pupae, of *Simulium decorum* that were on the wooden drop-logs of the Lake Sasajewun dam. This was the only time simuliid pupae were seen to be attacked. Crisp (5) stated that the larvae of *Chimarra* spp. attacked large numbers of *Simulium* larvae in the Northern Territories of the Gold Coast, but larvae of *Cheumatopsyche* spp. fed on algae and had little or no effect on *Simulium* larvae.

TABLE II

Summary of observed caddis-fly predation on black flies in Algonquin Park, Ontario, during the summer of 1955

Predators	Prey	Date	Locality
Hydropsychidae			
Diplectrona (prob.) *modesta* Banks	*Simulium venustum*	June 2	S. Madawaska River, S. of airport
Hydropsyche sp.	*Simulium venustum* *Simulium vittatum*	June 25	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Cheumatopsyche sp.	*Simulium venustum* *Simulium vittatum*	June 25	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Hydropsyche sp.	*Simulium venustum* *Simulium vittatum* *Simulium decorum*	June 25	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Hydropsyche sp.	*Simulium vittatum* *Simulium venustum*	June 30	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Hydropsyche sp.	*Simulium venustum* *Simulium vittatum*	July 7	Falls, S. Madawaska River, S. of airport
Hydropsyche sp.	*Simulium vittatum* *Simulium venustum*	July 10	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Hydropsyche sp.	*Simulium vittatum* *Simulium venustum*	July 10	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Hydropsyche sp.	*Simulium decorum*	July 14	Rapids, N. Madawaska River, ¼ mile S. of Lake Sasajewun
Cheumatopsyche sp.	*Simulium decorum*	July 17	Wooden drop-logs, Lake Sasajewun dam
Philopotamidae			
Chimarra sp.	*Simulium decorum*	July 13	Wooden drop-logs, Lake Sasajewun dam
Chimarra sp.	*Simulium decorum*	July 17	Wooden drop-logs, Lake Sasajewun dam
Limnephilidae			
Neophylax sp.	*Eusimulium euryadminiculum*	May 31	Small stream on N.W. shore of Lake Opeongo
Rhyacophilidae			
Rhyacophila fuscula (Walker)	*Simulium venustum*	May 21	Small tributary of N. Madawaska River, ¼ mile N.E. of Lake Sasajewun

On a number of other occasions during the summer of 1955, caddis-fly larvae were observed feeding on black-fly eggs and larvae; these observations are summarized in Table II. The predatory habits of certain species of caddis-fly larvae on simuliid larvae has been reported by a number of other workers (6, 7, 8, 13, 15, 16 ,17, 18, 19, 24, 29).

Hymenoptera

Formicidae

Ants were observed to prey on adult black flies on three occasions during the summer of 1955. The first occasion was on June 16, along the banks of the South Madawaska River, south of the airport, when several ants of the species *Myrmica emeryana* Forel were observed chewing on females of *Simulium venustum* and *Simulium decorum*. The ants were on sunny rocks near the edge of the stream and had probably caught the newly emerged flies as they were resting on the rocks drying their integuments.

The other two occasions of black-fly predation by ants took place on the walls of the sluiceway and on the wooden drop-logs of the dam. On July 11, at 8.10 p.m., shortly after sundown, two horizontal lines of *Lasius neoniger* Emery were observed on the cement walls of the sluiceway. The lower line consisted of individuals returning to their nest with prey, while the upper line, which was about 2 in. above the lower line, consisted of individuals returning from the nest to the "hunting ground". The foraging ants would crawl along the wooden drop-logs and wait for flies to land. Females of *Simulium decorum* were ovipositing into the thin sheet of water where it flowed over the drop-logs, and some landed to oviposit on moist portions of the logs. Other female flies landed on the logs apparently to rest after ovipositing. Such females proved to be easy prey for the waiting ants which quickly moved forward to seize the flies and carry them off.

Of the ants collected, individuals were found which carried one male that was still in pupal form but removed from the pupal skin, one female whose abdomen contained mature eggs and a mermithid nematode parasite, and one complete female with mature eggs. Several other ants were found which carried various parts of female flies.

Similar observations were made at the sluiceway on July 14. Numerous females of *Simulium decorum* that had recently oviposited were resting or dying on the cement walls and wooden drop-logs, and were easily caught by the ants (*Lasius neoniger*). One ant was observed that had hold of a female fly by the hind leg and was slowly dragging her backwards. The fly resisted by grasping and trying to hold on to small twigs and empty pupal cases as she was pulled along, but her efforts were in vain. Both the ant and the fly were collected and placed in alcohol to end the tragedy.

Crisp (5) mentioned that ants may attack exposed eggs, larvae, and pupae of *Simulium damnosum* Theobald in the Gold Coast of Africa.

Odonata

On the morning of May 27, while collecting immature stages of *Simulium venustum* from shallow rapids at the edge of a lake-like area of the North Madawaska River, we observed three naiads crawling over the rocks and devouring black-fly larvae. Two of the naiads were dragon-fly nymphs, *Aeshna umbrosa* Walker (Aeshnidae), and the other a damsel-fly nymph, *Agrion maculatum* Beauvois (Agrionidae). In each instance, the nymphs approached the simuliid larvae from the downstream side, and quickly seized and devoured their helpless prey.

Adult dragon flies, and occasionally damsel flies, were observed to fly in a darting manner above the rapids of a stream and feed on newly emerged or ovipositing adult flies. A number of other authors (3, 4, 5, 6, 7, 8, 10, 24) have reported that various species of Odonata preyed on black flies.

Araneae

Adult black flies were frequently observed trapped in, and were collected from, the webs of several species of spiders. The authors, on several occasions, have watched spiders pounce upon and consume black flies at nearly the same instant they were trapped. More often, adults were found wrapped in characteristic garments of silk, stored for future consumption.

Arhynchobdellida

Leeches were often observed on rocks in association with black-fly larvae and pupae. A number of leeches, *Haemopis marmorata* (Say) (Hirudidae), were observed on the rocks of the falls of the South Madawaska River, on July 7, but only one was seen to feed on black-fly larvae. Species of *Haemopis* are known to feed on small invertebrates or dead animal matter (20), and in some regions these or other species of leeches may possibly be active predators of immature black flies.

Other Possible Predators

Cameron (4) and Crisp (5) reported that the nymphs of certain species of May flies and stone flies were formidable enemies of black-fly larvae and may play important roles in reducing their numbers. In Algonquin park, many species of May flies and a few species of stone flies were observed in streams associated with black-fly larvae, but none were ever observed to be predaceous. In some areas, May-fly and stone-fly nymphs, as well as the nymphs and larvae of other aquatic insects, could very well be active and perhaps important predators of simuliid larvae and pupae.

Acknowledgments

We wish to acknowledge the assistance of the following specialists in the taxonomy of the various groups of insects who kindly confirmed or made identifications for the senior author: J. G. Chillcott (Empididae) and J. R. Vockeroth (Dolichopodidae), Entomology Research Institute, Research

Branch, Department of Agriculture, Ottawa, Ontario; C. W. Sabrosky (Ephydridae), Insect Identification and Parasite Introduction Research Branch, United States Department of Agriculture, Washington, D.C.; G. B. Wiggins (Trichoptera) and E. M. Walker (Odonata), Division of Zoology and Palaeontology, the Royal Ontario Museum, Toronto, Ontario; and, A. W. Grundmann (Formicidae), Department of Zoology, University of Utah, Salt Lake City, Utah.

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VARIATION, SEXUAL DIMORPHISM, AND MATURITY IN A QUEBEC POPULATION OF THE COMMON SNAPPING TURTLE, CHELYDRA SERPENTINA[1]

JAMES E. MOSIMANN AND J. ROGER BIDER

Abstract

An analysis was made of a sample of 55 snapping turtles, *Chelydra serpentina*, from a single locality in southern Quebec. The variation studied is regarded as being representative of that of a single population of the species. Analysis of covariance was used in studying dimorphism in various measurements. Conclusions reached are: (*a*) the sex ratio is one; (*b*) males grow to a larger size than females; (*c*) males and females become mature at a carapace length of about 200 millimeters; (*d*) the ratio precloacal distance over posterior lobe of the plastron gives a practical index for sex determination in adults; (*e*) there is a set of small but interrelated changes correlated with a greater precloacal distance in the male: These are that males have the plastron shifted forward slightly, have the bridge slightly longer relative to plastron length, and have the posterior lobe of the plastron slightly shorter relative to plastron length; (*f*) the precloacal length of the male (which area accommodates the penis) grows relatively faster than carapace length; the precloacal length of the female grows at the same relative rate as carapace length; (g) there is virtually no dimorphism in shell dimensions nor in head width.

Introduction

The common snapping turtle, *Chelydra serpentina*, of eastern North America, although well known popularly speaking, has been the subject of few concentrated morphological studies of variation. Only recently Richmond (13) has clarified the taxonomic status of the Florida snapping turtle, *C. osceola*, an animal previously thought to be a very slightly (if at all) distinct subspecies of the common snapping turtle. Now tentatively regarded as a species, *C. osceola* is at the least a well-marked race. The possibility of such a reversal in taxonomic judgment concerning animals as conspicuous and common as snapping turtles reflects the lack of detailed knowledge of their morphological variation, both ontogenetic and geographic. Much of this lack stems from difficulty in preservation due to the large size attained by these turtles. Due to generous support from the National Research Council, we were able to collect and preserve in its entirety a series of *C. serpentina* from southern Quebec during the summer of 1956. The present report is based on an analysis of this series.

Our purpose in this work is: *first*, to study sexual dimorphism in morphological characters involving the shell, the tail, and the head, all in relation with size at which sexual maturity is attained; and *second*, to provide a practical external means of determining sex in these animals. The present study is a continuation of a program by Mosimann (10, 11) of studies of variation within local populations of turtles, the eventual purpose being a general examination of the geographic variation of the intrapopulation variation itself.

[1]Manuscript received July 14, 1959.
Contribution No. 47 from the Department of Biology, University of Montreal, Montreal, Quebec.

Material and Habitat

Material

A series of 55 turtles was collected at intervals from May 21 to August 27, 1956. Of these, 53 were taken from a 4-mile stretch of the Pike River, 2½ miles from its mouth, where it enters the northeastern corner of Missisquoi Bay, a part of Lake Champlain in Missisquoi County, Province of Quebec. In addition, two egg-laden females were from an overflow area on the northern edge of the bay about 3 shoreline miles from the entrance of Pike River. All individuals are thus from a restricted locality representing an uninterrupted stretch of water in Missisquoi County. As it is apparent that the locomotor abilities of an individual are such as to permit it easily to traverse the entire area during its lifetime, we therefore see no evident reason why our sample might not represent a single deme. Nevertheless, the use of the word population in this report connotes no more than a judgment concerning the area of collection and the locomotor abilities of the animal (10, p. 6). Specimens are in the Department of Biology of the University of Montreal.

Habitat

The following account is descriptive of conditions existing during the period of collection. Water was higher in May, with standing water in overflow areas. A sawmill dam at Notre Dame de Stanbridge (2 miles upstream from the Pike River collecting area) regulated water flow so that depth during the late summer was relatively constant. Measurements of the length of the river were made from a National Topographic Sheet (Lacolle, 1:50,000, 31-H-3) of the Department of Mines and Technical Surveys, Ottawa. On this map two dams are shown in the 4-mile-long area of collection itself. These have not been in existence for a considerable time. Terminology for particle sizes of sediments follows that of Wentworth (16, p. 381) as presented in the somewhat simplified table of Garrels (6, p. 45). Reference to the original article rather than that of Garrels is particularly informative because of photographic illustrations in the former.

The 4-mile stretch of river was divisible into three sections as follows: *Upstream section*, 2½ miles in length, about 150 feet at its widest, 75 feet at its narrowest. Generally clear and shallow, 1 to 3 ft deep, with scattered deeper pools. Characterized by abundant exposed boulders resting on bedrock or on a pebble and cobble bottom. Wading possible throughout entire length, boating impossible. Aquatic plants nearly absent, limited to stream edge or present only as drifting masses fortuitously attached to scattered emergent boulders. Markedly lotic, few lentic situations. *Middle section*, ½ mile in length, about 100 feet wide, water clear. Depth variable, in places very shallow (6 in. deep), running over bedrock, in others, up to 6 ft deep in pools. Relatively few exposed boulders. Bottom, bedrock and silt. Wading impossible in some areas, boating impractical. Aquatic vegetation more abundant. Lotic but with many localized lentic areas. *Downstream section*, 1 mile in length, about 175 feet wide. Water less clear. Depth 4 to 8 feet.

Bottom uniform silt. No emergent boulders. Aquatic vegetation very abundant in areas. Wading impossible, boating necessary. Predominantly lentic.

The division between the upstream and middle sections was not so clearly marked as that between the middle and downstream sections. Between the latter two a sharp change in stream gradient produced an abrupt shift from a predominantly lotic to a predominantly lentic environment. At this point the river becomes an estuary of the bay, although 3½ miles from the bay proper. The middle section represents the maximum penetration of the bay's influence during the flood conditions of early spring.

Snapping turtles were collected from all three sections of the 4 miles described. Further upstream no turtles were seen despite intensive work. The habitat further upstream was not noticeably different from that of the upstream section. Snappers were observed along the entire river below the collecting area as far as and into the bay itself.

In contrast, painted turtles, *Chrysemys picta*, were limited to lentic situations. They were common in the downstream section, occasional in the middle section, and virtually absent from the upstream section. A series of 73 individuals was collected, of which less than 10 came from the middle section, none from further upstream. (Only two were seen during the entire period in the upstream section.)

Methods

Collecting Techniques

A diversification of collecting techniques was necessary to insure a balanced representation of size classes in the sample. The techniques used may be divided as follows: (1) *Hand-collecting of basking turtles.* With binoculars, basking turtles were located on boulders. One of us waded towards the boulder while the other remained on the bank scanning the clear water for indications of the fleeing animal. The turtle was invariably discovered motionless under a near patch of drifting aquatic plants. The method was useful in the upstream and middle sections on sunny days and produced large turtles but few small ones. (2) *Hand-collecting of partially immersed turtles.* With binoculars, noses of turtles were located in shallow water. Approach was then made and the turtle was usually found motionless near the point of disappearance. This method was useful in the upstream and middle sections on all days and particularly effective at dusk. Large to very small turtles were secured. Five of six turtles in the range of 60–110 mm carapace length were taken at dusk. (3) *Muddling.* Concealed turtles were located with a probe or with feet, usually in areas of turbid water. This method was particularly effective in flooded areas adjoining the downstream and middle sections during the spring. Large to small turtles were thus secured. (4) *Net-collecting from boat.* With binoculars, noses were sighted in shallow to deep water. The turtles were subsequently netted from the boat. Used in the downstream section exclusively, this method was effective on all days and produced medium to small turtles. (Large turtles were seen but not netted.)

No trapping was attempted. Night-collecting with a spotlight was not successful, no snapping turtle activity being revealed.

Measurements

The following measurements were used in studying dimorphism (Fig. 1): *carapace length* (CL), the median length of the carapace along a straight line (not along the curve of the back); *carapace width* (CW), the maximum straight line width of the carapace; *carapace height* (CH), the maximum straight line height of the shell, measured as perpendicular to the plastron; *plastron length* (PL), the median length of the plastron along a straight line; *precloacal distance* (PRE-CLO), the distance from the median posterior margin of the plastron to the anterior edge of the cloaca (also called precloacal length); *postcloacal distance* (POST-CLO), the distance from the median posterior edge of the cloaca to the tail tip (also called postcloacal length); *anterior to the posterior end of the plastron length* (APPL), the distance along a straight line from an anterior point (marking the intersection with this line of a perpendicular dropped from the median anterior border of the carapace) to the median posterior margin of the plastron; *head width* (HW), the width of the head at the level of the tympanum; *bridge length* (BL), the maximum length of the scute covering the bridge between the inframarginals and the plastron proper (the scute called the abdominal by Boulenger (1)); *bridge width* (BW), the narrowest width of the bridge; *posterior lobe of the plastron length* (POST-L), the mid-line length of the last two plastral scute pairs. This corresponds roughly to the length of the plastron posterior to the bridge.

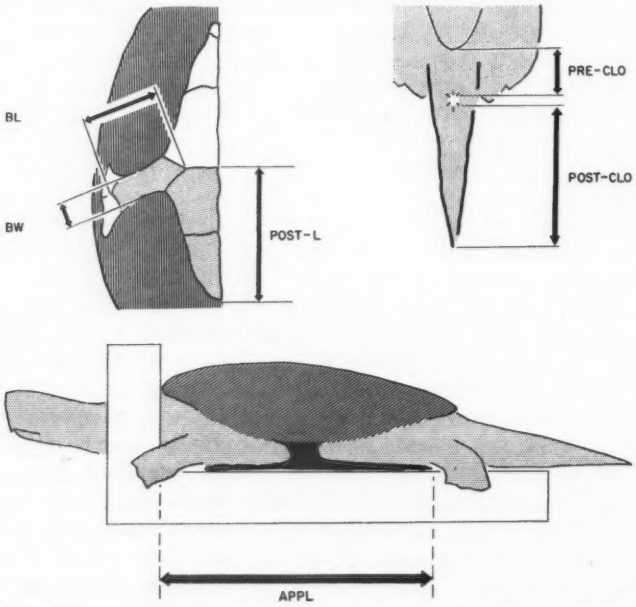


FIG. 1. Measurements used in the present study. Only the less standard measurements are illustrated. Abbreviations are as follows: BL, bridge length; BW, bridge width; POST-L, posterior lobe of the plastron length; APPL, anterior to the posterior end of the plastron length. Drawings are diagrammatic and not intended to indicate proportions.

Carapace length, carapace width, and carapace height are all objective measurements taken from hard surfaces and can be measured with considerable accuracy. Of the three, carapace height is the most difficult to obtain and is probably the least accurate. Plastron length in the snapping turtle is a measure taken from a relatively hard surface (consisting of cartilage and bone) and can also be measured with considerable accuracy. The measurement APPL was taken from two objectively determined points, but was subject to possible variation depending on possible changes in the angle between carapace level and plastron level due to degree of inflation or deflation of the preserved animal. We do not consider this possibility of critical importance here. Head width was taken at a point where it is possible to press the calipers against skin in contact with the skull. Thus there is no error due to presence of variable muscle masses in different individuals. The tail measurements, pre- and post-cloacal distance, are taken from soft parts and as such are more subject to variation due to preservation than the above measurements.

Long length measurements were taken with calipers improvised from a meter stick and two right angles. Smaller measurements were made with vernier calipers. Tail measurements in all cases were taken with dividers. APPL was measured using a right-angle device. Measurements were recorded to the nearest millimeter, with the exceptions of head width, bridge length, and bridge width, which were recorded to the nearest 10th of a millimeter.

Statistical Methods and Analysis

A presentation of the analysis of covariance tests used in this paper may be found in Snedecor (14, p. 318 *et seq.;* 15, p. 394 *et seq.*). Snedecor's test for adjusted mean differences is the position test in this paper. His test for regression coefficients (14, p. 327; 15, p. 398) corresponds to the slope test here. A sample analysis is given in Table II.

We have applied slope and position tests in the analysis of each bivariate relation studied. An assumption underlying the position test is that parameter slopes of the regressions being tested are equal. Where slopes were clearly significantly different we have nevertheless included the position tests even though interpretation of significance in the latter is rendered ambiguous. It might be noted that a significant result can be obtained for a slope test without corresponding significance in the position test (e.g., plastron length and carapace length). In any case, where slope tests give significant results our judgment is based on these rather than on the corresponding position tests.

There is no independent variable among those studied here and consequently the applications of regression and covariance analysis are not valid in a strict sense. The question asked by us of the data is "are males different from females" and this not a question only of estimating one variable from known values of another. Thus for every bivariate relation examined we have chosen each variable in its turn as independent. As a result, variances tested here are measured from lines based on least squares of y deviations as well as

from lines based on least squares of x deviations. Even so, there remains the problem of bias in our sample regression slopes as estimates of parameter slopes. Kenney and Keeping (7, pp. 213–14) have presented a model for regression when both variables are subject to error, and McIntosh (9) in a further utilization of this model has studied the effects of such error in the analysis of covariance of skeletal measurements of mice (*Peromyscus*). For his data McIntosh concluded that errors in the independent variable did not affect his tests to the point of modifying conclusions reached. We have not judged it fruitful in this case to use McIntosh's method.

In the light of the foregoing it seems desirable for us to make explicit our conception of the use of covariance analysis in the present case. We think that this technique is simply a tool which though admittedly imperfectly suited to the task at hand, nevertheless is a sufficient improvement over no tool at all to warrant its use with considerable profit. First, the least squares aspect of the analysis has descriptive validity. Second, the significance tests used permit evaluation *as if* the distributions behind the samples here were bivariate normal (implying linear regressions of y on x and of x on y and variance homoscedasticity about both). Even though we might suspect that such is not the case the test may serve as a useful basis for judgment. For example, a non-significant result obtained given the above assumptions will certainly lead the biologist to examine his position (and the assumptions) carefully if he thinks nevertheless that an observed difference is biologically real.

In addition, we think that the following points should be made explicit. Conclusions in the present study are based on three partially distinct bases for judgment:

(1) visual observation and study of the specimens concerned,
(2) results of the covariance analyses based on the numerical data,
(3) graphical study of the bivariate relations concerned.

We do not hesitate to state that (1) and (3) are of equal importance with (2) in this study. Yet (1) and (3) alone do not replace (2). In short, significance tests themselves are only aids to the judgment of the worker. They are valuable in as much as they aid the worker in making a judgment and in as much as they can be used to convey in approximate terms the degree of assurance which the worker attaches to such-and-such a result.

The above discussion reveals the subjective element in our analyses. We think that to hide this subjective element under elegant statistical terminology would in itself constitute a more serious error.

In our tests we have compared all males (juveniles and adults) with all females (juveniles and adults). Juveniles of both sexes are similar and consequently dimorphism might be more easily observed in comparing adults alone. However, males grow larger than females and the comparison would still call for covariance analysis. Over the short range afforded by adult males and females the estimation of regression slopes would be correspondingly less accurate than that made by including juveniles. Of more importance is our

interest in the development of dimorphism as well as in the final form of the differences between sexes. For this the analysis with juveniles included is clearly preferable.

It should be noted that since juveniles of both sexes are similar, differences are to be expected in the slope test if dimorphism occurs.

Sex Ratio, Maturity, Size

Sex Ratio

In the 55 turtles, there are 28 females and 27 males. Thus the sex ratio is clearly not significantly different from one. In 151 Michigan snapping turtles discussed by Lagler and Applegate (8) there were 77 females and 74 males. Lagler (personal communication) informs us that there was no conscious bias for a given sex in collecting this sample. Thus on the basis of these two samples we assume the sex ratio to be one.

Maturity

Sexes were determined by dissection and in young individuals by subsequent microscopic examination of the gonads. Weights (Table I) were obtained for all male right testes and for all female right ovaries plus oviducts. Weights were of preserved organs. Most of the specimens were preserved immediately upon death by injecting the body cavity under pressure sufficient to distend limbs and neck, and consequently gonads were in a good state of preservation. However, two specimens had been dead for some time before preservation, and correspondingly the gonads were poorly preserved. Although weights in this study are subject to variation due to extraneous factors, they are more than accurate enough to be of considerable aid in delimiting size at maturity.

For males and females of comparable size (even at the smallest sizes represented here) female organs are considerably larger than those of the male (Table I). This fact was determined only after the identification of sex from the gonadal tissue, and was not in itself used in the determination.

Maturity in male specimens was determined by detection of sperm in smears from the testis (Table I). For females the procedure was as follows. Two females contained oviducal eggs and were clearly mature. Follicles remaining in the ovaries of these two were presumably capable of producing eggs during the next season. Thus the maximum diameter of the largest follicle in each of these turtles was measured and used as a standard in determining maturity. Any female with follicles of larger or equal size is regarded as mature in the sense that she would probably have been capable of laying eggs in the following season. The reasoning and techniques here follow those of Cagle (3, 4).

We conclude that maturity occurs in both sexes at a carapace length of about 200 millimeters. Further work with more specimens in the critical size range will doubtless render this more precise and may indicate that the range of size at which females mature has a somewhat lower limit than that of males.

A single specimen male No. 1151 (CL 393) had small testes relative to his total size. Unfortunately this specimen was dead for several days before preservation and his internal organs (including the testes) are very poorly preserved. His tail is quite short relative to carapace length (Figs. 6, 7). We think there is the possibility that this individual was senile, but due to the lack of well-preserved specimens of this size, this remains speculation. In certain covariance analyses that are to follow we have performed tests both with and without this male.

TABLE I

Determination of maturity in males and females

NOTE: The specimens above the break in each column are regarded as mature. *Males:* the symbol "N" means testis examined and no sperm found. In addition, the weight and histological aspect of the testis was not that of a mature animal. The symbol "R" means the testis was examined, and no sperm found, yet the weight and histological aspect of the testis was that of a mature animal. In one case (CL 393) the testis was in a poor state of preservation. The symbol "—" indicates no examination was necessary, the animal being clearly immature. *Females:* the symbol "E" indicates the presence of well-developed oviducal eggs. The measurements of follicle diameter were made with vernier calipers, with the exception of that for the turtle with CL 200, whose measurement was made with an ocular micrometer. The turtle with CL 190 is nearing maturity.

Males			Females		
Carapace length (mm)	Testis weight ($g \times 10^3$)	Sperm presence	Carapace length (mm)	Ovary and oviduct wt. ($g \times 10^3$)	Maximum follicle diameter (mm)
393	8951	R	281	142400 (E)	9.1
345	19947	S	276	209700 (E)	10.0
297	11443	S	258	40000	13.0
297	8125	S	258	91800	13.8
283	3162	R	257	131600	17.3
275	3276	S	238	48200	18.0
271	6945	S	237	33400	12.5
270	5560	S	231	45400	13.2
262	4455	R	225	32600	11.6
244	1477	R	224	16700	16.0
227	1096	R	223	40600	16.8
210	662	S	219	34000	16.4
206	430	N	216	13200	4.5
205	545	N	200	1300	1.5
176	77	N	190	8600	9.5
170	198	N	187	570	—
170	163	N	159	540	—
150	58	—	157	318	—
140	36	—	154	246	—
125	26	—	151	128	—
121	29	—	141	132	—
120	26	—	131	169	—
120	14	—	118	79	—
118	23	—	114	41	—
106	12	—	99	75	—
87	6	—	90	36	—
86	5	—	67	27	—
			45	3	—

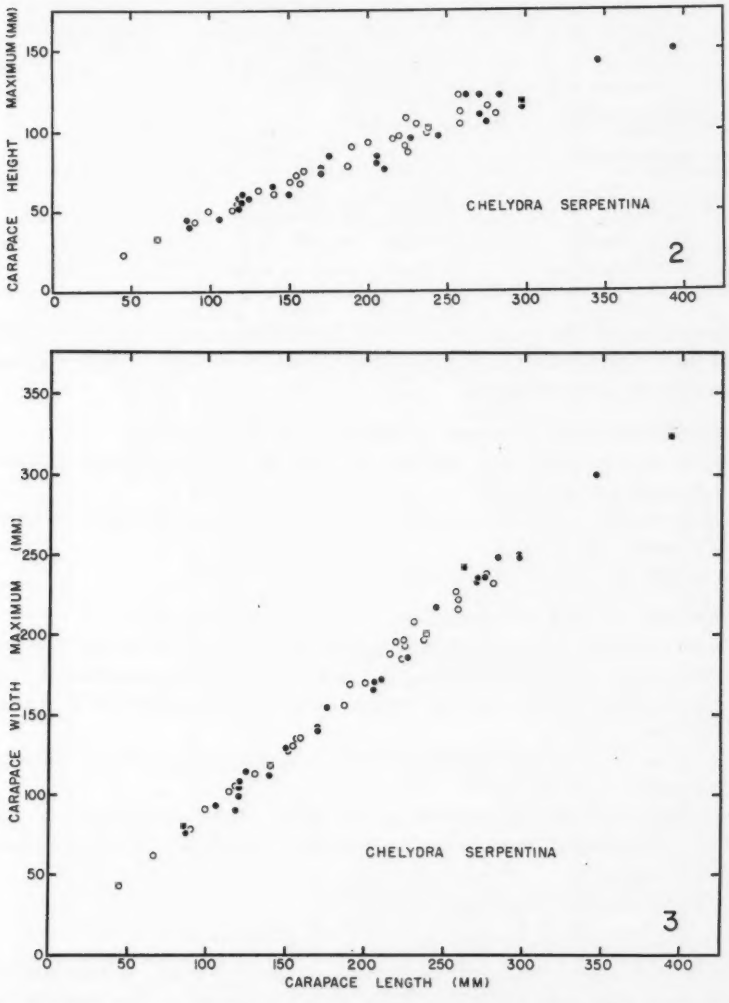
Size

In discussing *C. serpentina*, Pope (12, p. 73) reports: "The female is said to attain a larger size than the male...." Carr (5, p. 63) makes a qualified statement to the same effect: "The male is perhaps a trifle smaller...."

In our sample, males constitute the larger sex. The largest male is 393 mm in carapace length, the largest female is 281 mm. There are five males larger than the largest female. This result contradicts the statements quoted above.

Sexual Dimorphism

In the present analysis the data are (with the exception of graphs involving precloacal length) quite rectilinear in aspect. Further heteroscedasticity (to judge visually) is not excessive in most of the relations. In the following summaries the analysis based on the square of the deviations on y is given first, that based on the square of the deviations in x is given second.



FIGS. 2 and 3. The relation of carapace height and carapace length (Fig. 2) and of carapace width and carapace length (Fig. 3). Males are solid circles; females, hollow circles.

Carapace Height (*y*) *vs. Carapace Length* (*x*) (Fig. 2, Tables II and III)

y on *x:* neither slope nor position test reveals any significant difference between males and females.

x on *y:* neither slope nor position test reveals any significant difference between males and females.

TABLE II

Covariance analysis of carapace length and carapace height

		Length deviations for given heights			Height deviations for given lengths		
	D.f.	S.S.	M.S.	*F*	S.S.	M.S.	*F*
(1) From single best-fitting line for both groups	53	12247.550			1823.800		
(2) Pooled from best-fitting parallel lines	52	11619.121	223.445		1746.573	33.588	
(3) Pooled from best-fitting individual lines	51	11241.715	220.426		1690.146	33.140	
Difference (2—3) for slope test	1	377.406	377.406	1.71	56.427	56.427	1.70
Difference (1—2) for position test	1	628.429	628.429	2.81	77.227	77.227	2.30

Examination of the graph shows no marked difference in trend, but, over part of the range, female points are higher than those of males. No dimorphism is shown in the present sample.

Carapace Width (*y*) *vs. Carapace Length* (*x*) (Fig. 3, Table III)

y on *x*: neither slope nor position test reveals any significant difference between males and females.

x on *y:* neither slope nor position test reveals any significant difference between males and females.

The graph shows no difference in trend.

Plastron Length (*y*) *vs. Carapace Length* (*x*) (Fig. 4, Table III)

y on *x:* the slope test gives significance at the 5% but not at the 1% level. The position test reveals no significant difference. If the largest male No. 1151 (CL 393) is dropped from the calculation, no significant difference is revealed by either test.

x on *y:* neither slope nor position test reveals any significant difference between males and females.

Examination of the graph shows no difference. There may be a very slight tendency for males to have a shorter plastron, but this is not demonstrable with this sample.

No dimorphism in this relation is shown.

APPL (*y*) *vs. Carapace Length* (*x*) (Fig. 5, Table III)

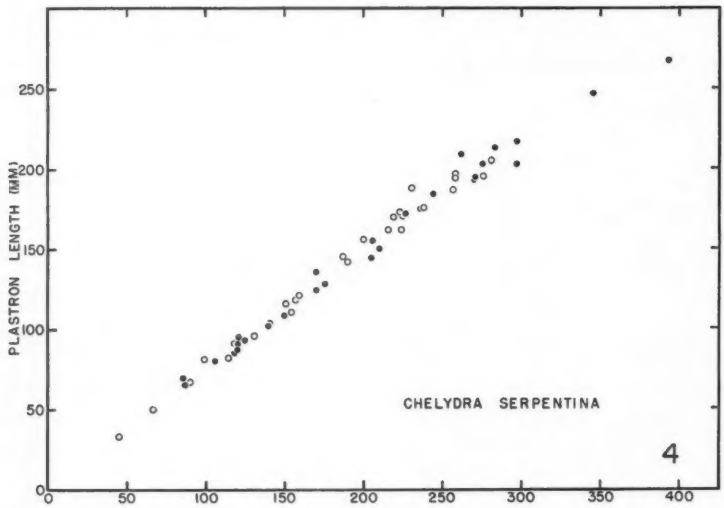
y on *x:* the slope test is just significant at the 1% level. Eliminating the largest male, the *F*-value for the slope test is increased. The position test, both with and without No. 1151, gave significance at the 5% but not the 1% level.

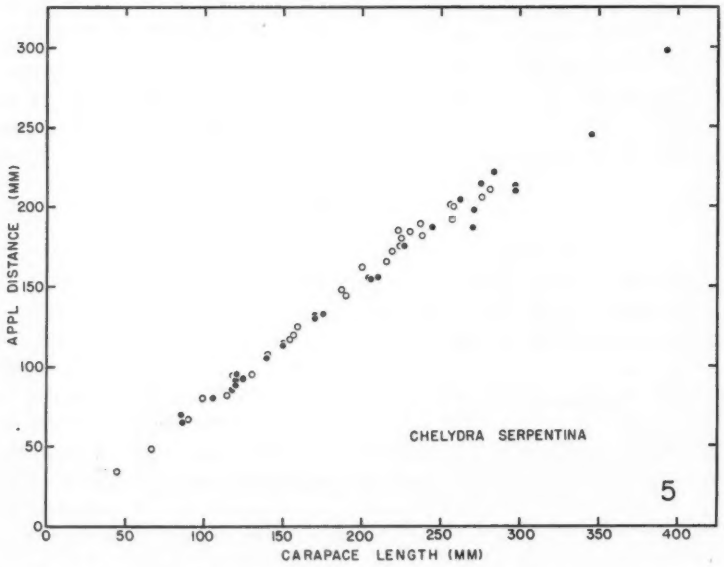
TABLE III

Analyses of covariance

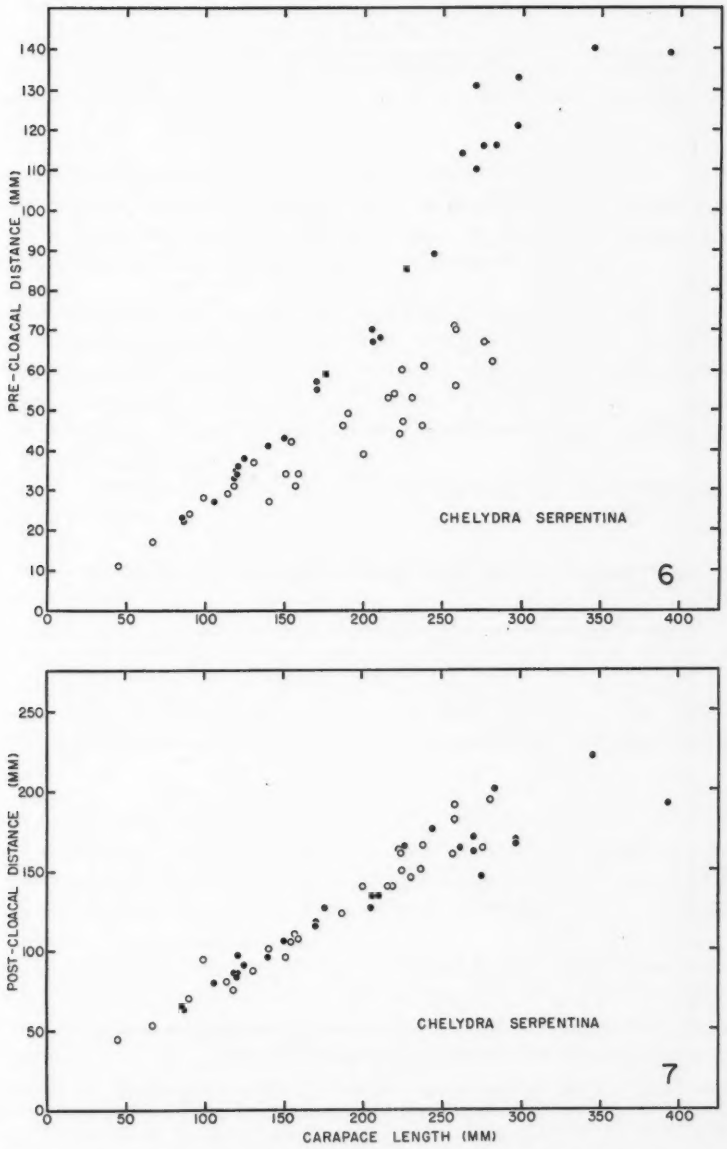
		F values for slope test						*F* values for position test					
		Complete sample			Male 1151 excluded			Complete sample			Male 1151 excluded		
X	*Y*	D.f.	*X* on *Y*	*Y* on *X*	D.f.	*X* on *Y*	*Y* on *X*	D.f.	*X* on *Y*	*Y* on *X*	D.f.	*X* on *Y*	*Y* on *X*
CL	CH	1,51	1.71	1.70				1,52	2.81	2.30			
CL	CW	1,51	0.51	0.36				1,52	1.12	0.95			
CL	PL	1,51	3.93	4.23*	1,50		1.49	1,52	3.37	3.01	1,51		2.42
CL	APPL	1,51	6.88*	7.18**	1,50	8.68**	9.50**	1,52	7.15**	6.66*	1,51	7.16**	6.85*
CL	PRE-CLO	1,51	45.71**	76.00**				1,52	33.77**	54.24**			
BL	BW	1,51	3.98	2.68				1,52	1.28	0.33			
CL	POST-CLO	1,50	3.77	6.57*	1,49		2.54	1,51	2.85	2.18	1,50		1.65
PL	POST-L	1,51	4.80*	4.38*	1,50	4.35*	4.25*	1,52	4.96*	4.65*	1,51	4.51*	4.35*
PL	BL	1,51	3.58	6.02*	1,50	4.90*	7.52**	1,52	5.75*	6.97*	1,51	6.17*	7.12*
CL	HW	1,51	4.00	4.25*	1,50		2.44	1,52	1.88	2.19	1,51		1.74

NOTE: For CL vs. APPL, interpolation gives 7.15 and 7.16 as the critical 1% values of *F* at 1,52 and 1,51 degrees of freedom respectively. One female with a broken tail tip is excluded from the analysis of CL vs. POST–CLO.

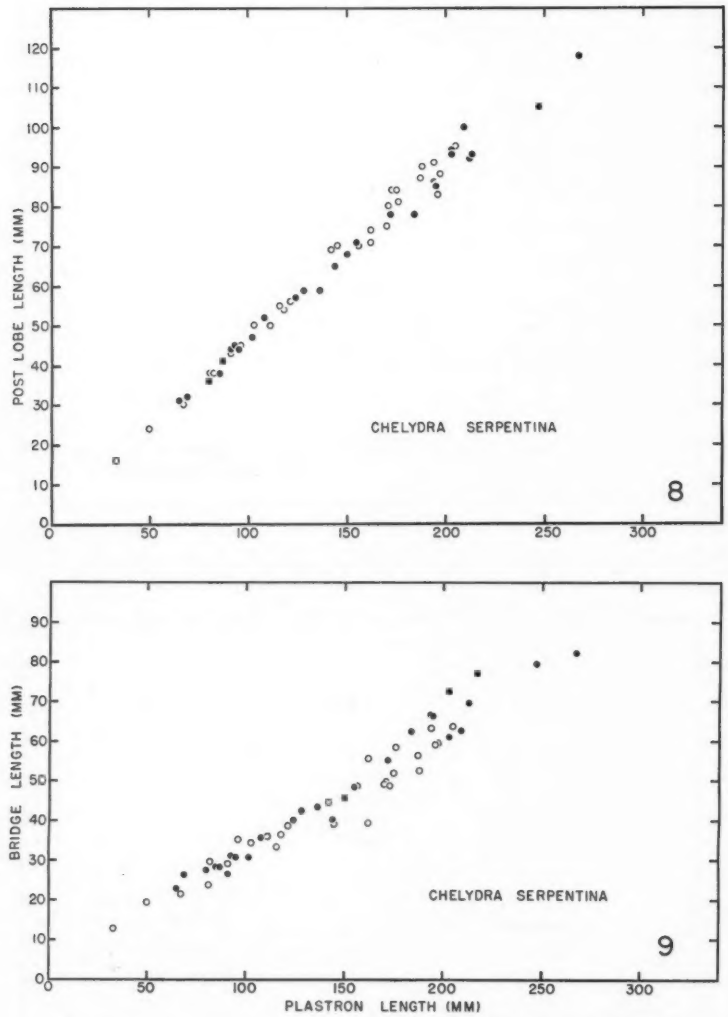




FIGS. 4 and 5. The relation of plastron length and carapace length (Fig. 4) and of APPL and carapace length (FIG. 5.). Males, solid circles; females, hollow circles.



FIGS. 6 and 7. The relation of precloacal (Fig. 6) and of postcloacal (Fig. 7) distance and carapace length. Males, solid circles; females, hollow circles. In Fig. 7, one female with a broken tail tip is not included in this graph.



FIGS. 8 and 9. The relation of posterior lobe of the plastron length and plastron length (Fig. 8) and of bridge length and plastron length (Fig. 9). Males, solid circles; females, hollow circles.

x on y: the slope test reveals significance at the 5% level, but without male 1151 the significance is at the 1% level. Position tests both with and without male 1151 are borderline at the 1% level.

Examination of the graph shows that although there is considerable overlap between the male and female scatters there is a small but clear difference in trend.

Precloacal Distance (y) *vs. Carapace Length* (x) (Fig. 6, Table III)

y on x: both the slope and position tests give highly significant results.

x on y: both the slope and position tests give highly significant results.

Examination of the graph shows a very marked dimorphism.

Postcloacal Distance (y) *vs. Carapace Length* (x) (Fig. 7, Table III)

y on x: the slope test is significant at the 5% level. If male 1151 is eliminated the slope test is non-significant. The position test is neither significant before nor after elimination of No. 1151.

x on y: neither slope nor position test reveals any significant difference between males and females.

Graphic examination reveals considerable variation in this relation. No difference in trend is noted.

Posterior Lobe (y) *vs. Plastron Length* (x) (Fig. 8, Table III)

y on x: the slope and the position tests are significant at the 5% level both before and after the elimination of male 1151.

x on y: both the slope and the position tests are significant at the 5% level before and after elimination of male 1151.

Examination of the graph shows considerable overlap in the male and female scatters, but a marked tendency for female points to be higher than those of males.

Bridge Length (y) *vs. Plastron Length* (x) (Fig. 9, Table III)

y on x: the slope test is significant at the 5% level, but without male 1151 the significance is at the 1% level. The position test is significant at the 5% and almost at the 1% level. Without male 1151 the F value is virtually unchanged.

x on y: the position test is significant at the 5% level, both with and without male 1151. The slope test is not significant but when No. 1151 is eliminated from the sample, the slope becomes significant at the 5% level.

Examination of the graph shows a clear tendency for female points to be lower than those of males.

Bridge Width (y) *vs. Bridge Length* (x) (Fig. 10, Table III)

y on x: neither slope nor position test shows significance.

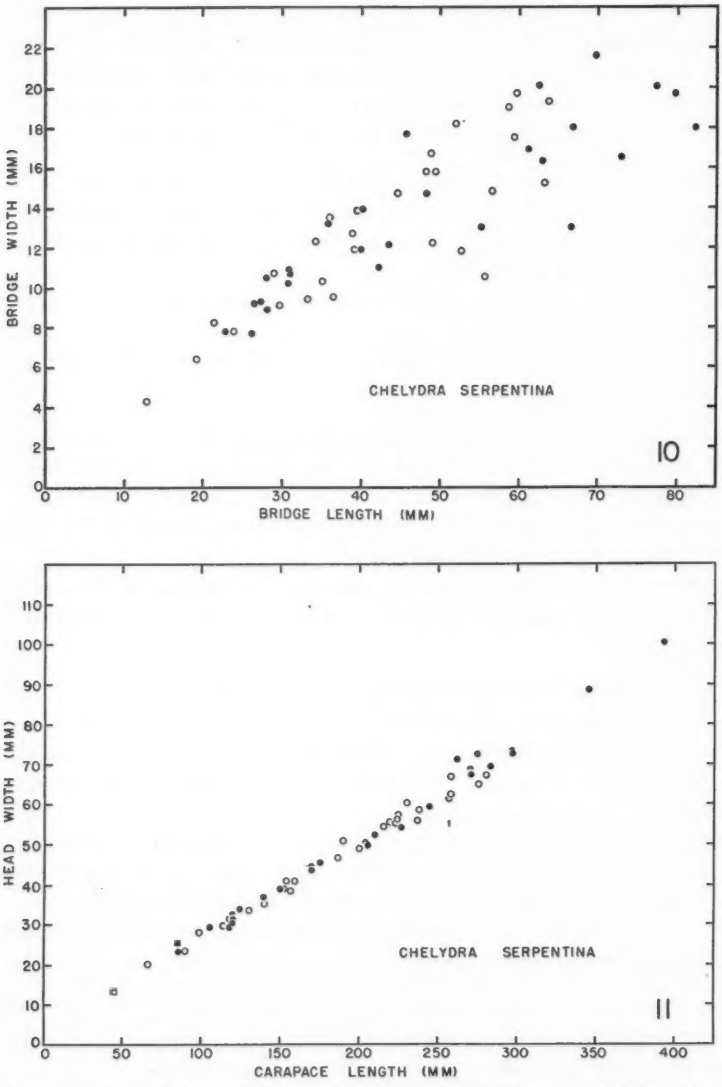
x on y: neither slope nor position test shows significance.

Examination of the graph reveals considerable variability in this relation.

Head Width (*y*) *vs. Carapace Length* (*x*) (Fig. 11, Table III)

y on *x*: the slope test is significant at the 5% level, but without male 1151 the test is not significant. The position test is not significant with or without male 1151.

x on *y*: neither slope nor position test shows significance between males and females, although the former test gives borderline results.



FIGS. 10 and 11. The relation of bridge width and bridge length (Fig. 10) and of head width and carapace length (Fig. 11). Males, solid circles; females, hollow circles.

Examination of the graph shows no consistent difference in trend between males and females. To observe whether some dimorphism occurs in adults would require more of the larger turtles of both sexes. No dimorphism is demonstrated here.

Relative Growth

The precloacal-distance–carapace-length relation has been shown to be strongly dimorphic in adults. The differential growth producing this difference was studied. As the relation showed considerable curvilinearity, regression lines were fitted to the logarithms of the data rather than to the original data. The reasoning behind the analysis of relative rates here is based on the previous treatment of Mosimann (11, pp. 146–151). Table IV summarizes the results. Calculations also follow procedures as outlined in ref. 11.

None of the female slopes are significantly different from unity; all male slopes are. The results show clearly that as an average the precloacal length in females increases at the same relative rate as carapace length. In males, precloacal length increases relatively faster than carapace length. In other words, as an average, precloacal length multiplies itself once each time that carapace length multiplies itself once in females. In males, precloacal length multiplies itself 1.4 times each time carapace length multiplies itself once.

The dimorphism in the tail therefore is due to the faster growth in the precloacal area of the male.

TABLE IV

Slopes of regression lines and of organic lines of correlation for the logarithms of precloacal distance (y) and the logarithms of carapace length (x). Plus and minus one standard error of slope is indicated. The slopes are estimates of relative growth rates. The reciprocal of x on y slopes is included for convenience of interpretation of relative rates. (The standard error of the y on x slope is the same as that of the organic slope.)

	Females	Males
$b_{y \text{ on } x}$	$.931 \pm .053$	$1.377 \pm .039$
b_{organic}	$.970 \pm .053$	$1.391 \pm .039$
$b_{x \text{ on } y}$	$.990 \pm .056$	$.712 \pm .020$
$1/b_{x \text{ on } y}$	1.010	1.404

Discussion and Conclusions

Relative to the striking sexual dimorphism often found in turtles, the sexes of *Chelydra* are remarkably similar. There is virtually no sexual dimorphism in shell dimensions, nor in head width.

The only reliable external indicator of sex is the distance from the plastron to the cloaca. This is relatively longer in adult males than in adult females. In specimens less than 200 mm in carapace length this character is not reliable.

The ratio precloacal distance over the posterior lobe gives a practical index for sex identification. Figure 12 shows the limits within which this can be used. In no female is this ratio over 86%. In most mature males the precloacal distance is longer than the posterior lobe and the ratio is greater than 100%. In no mature male is it less than 86%. Any specimen with the precloacal distance longer than the posterior lobe is a male.

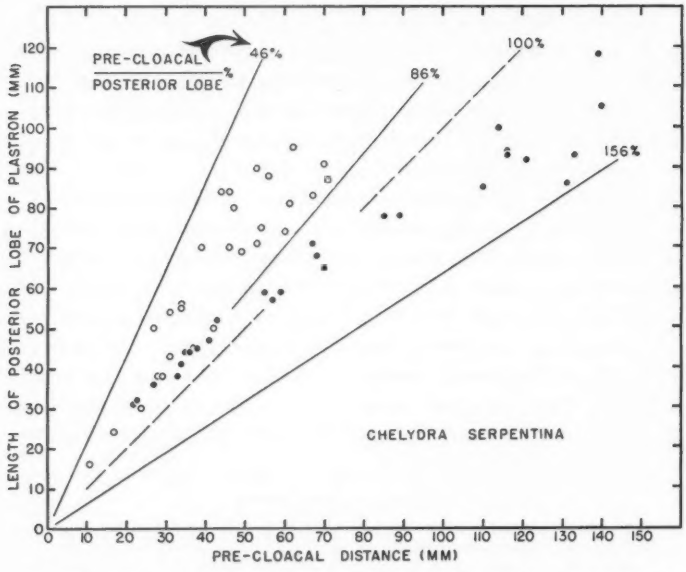


FIG. 12. The relation of posterior lobe of the plastron and precloacal distance showing sexual dimorphism.

The use of the above index is based on the relatively larger precloacal area in males and its efficiency is very slightly increased by the shorter posterior lobe of the plastron in this sex.

The tail as a whole is longer in males than in females, but this difference is due entirely to the relatively longer precloacal area of the male. There is no sexual dimorphism in the postcloacal distance. The longer precloacal distance in males might be a result of one (or a combination of) the following (cf. Fig. 13):

(*a*) a longer precloacal portion of the tail,
(*b*) a shorter plastron length relative to carapace length,
(*c*) a shorter posterior lobe of the plastron relative to plastron length,
(*d*) a forwardly shifted plastron.

Our data show that males have the plastron very slightly shifted forward (cf. CL vs. APPL) but that this is very small compared with the total difference in precloacal length. No difference in carapace length to plastron length is shown. The posterior lobe of the plastron is very slightly shorter in males

than in females. Again, however, this is very small compared with the total precloacal length difference. No sexual difference is noted in bridge length relative to bridge width; however, a small but clear difference exists between length and plastron length. Males have a relatively longer bridge on the average. This relates well to the forwardly shifted plastron in males. This may be the difference detected by the expert eye of Boulenger when he surmised (1, p. 22) that the bridge was broader in females than in males.

Although some of the above changes are small, they are clearly interrelated and all reflect the changes occurring in the precloacal area of the male turtle which is the region which accommodates the penis. Also shown is the fact that the difference in precloacal length is due to the male's more rapid precloacal growth relative to carapace length.

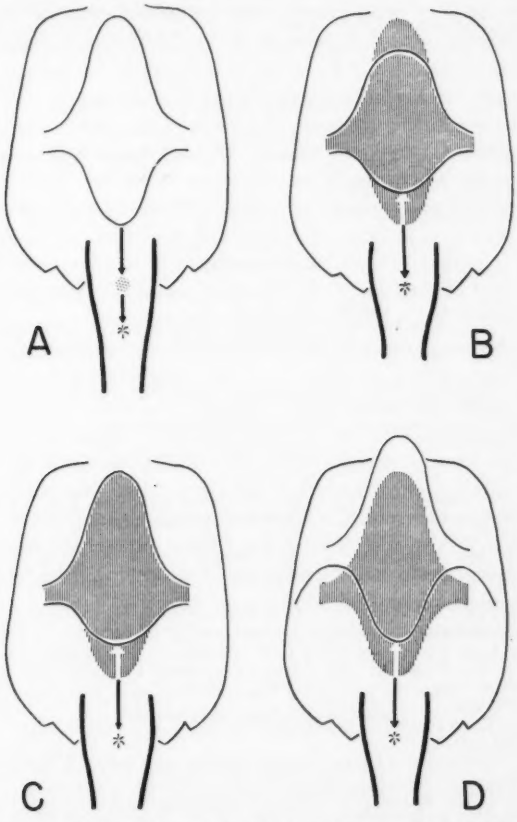


FIG. 13. Examples of possible ways in which a longer precloacal distance might be produced. The changes illustrated might be effective either singly or in combination. In (A) there is a longer precloacal region of the tail. In (B) there is a shorter plastron length relative to carapace length. In (C) there is a shorter posterior lobe of the plastron relative to plastron length. In (D) there is a forwardly shifted plastron. (Note that the bridge should elongate with this shift.) Drawings are diagrammatic and not intended to indicate proportions.

The excess growth of this area in the male is related to the development of the penis and could conceivably be stimulated by male hormones since Burns (2, p. 482) has stated in connection with sexual development in birds and mammals that "The erectile tissues, which largely determine the form of the penis, are stimulated by male and virtually suppressed by female hormone...."

Acknowledgments

We would like to express our appreciation to the Gessner and Phenix families of Pike River for kindly allowing us to collect turtles from their lands bordering the river. For pertinent comments, we are grateful to Drs. Jacques St-Pierre and Alexis Zinger, Center of Statistics, and to Miss Rita Carrière, Department of Biology, all of the University of Montreal. We would also like to thank the following, who kindly sent us information or data concerning size and sex in *Chelydra* from various localities: Mr. Roger Conant, Philadelphia Zoological Garden; Dr. John M. Legler, University of Kansas; Dr. Karl F. Lagler, University of Michigan; and Dr. Albert Schwartz, Albright College. We also wish to thank this journal's reviewer. Inclusion in the above list in no way implies agreement with the content of this paper, and sole responsibility naturally rests with the authors.

Research reported herein was supported by the National Research Council.

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A PHARMACOLOGICALLY ACTIVE AGENT IN THE REPRODUCTIVE SYSTEM OF INSECTS[1]

K. G. DAVEY[2]

Abstract

The opaque accessory secretion from the male reproductive system of insects has been characterized in various ways. It exerts a melanophorotropic effect on the melanocytes in the skin of the frog, *Rana pipiens*, a property which, among naturally occurring compounds, is shared only by the indolalkylamines. The secretion also increases the rate of beating of the heart of the cockroach *Periplaneta americana;* its activity in this respect is unaffected by heating or by incubating with the enzyme trypsin, but it is destroyed by the action of the enzymes monoamine oxidase, tyrosinase, and *o*-diphenol oxidase. The active principle is associated with particles from which it is slowly released into solution. It is tentatively concluded that the material is an *o*-dihydroxyindolalkylamine.

Introduction

In an earlier communication (4) the secretion in the opaque accessory gland in the male of *Rhodnius prolixus* was found to be responsible for the movements of the semen in the female by promoting contractions in the oviducts. The secretion acted through a peripheral nervous system which could function in isolation from the central nervous system. The secretion of similar glands, the utriculi majores of Miall and Denny (8), in *Periplaneta americana* also caused contractions in the oviducts of *Rhodnius*. This paper presents an account of experiments designed to investigate the nature of the active principle in the secretion.

An investigation of this sort requires that there be a reliable method for the assay of the material in question. The isolated oviducts of *Rhodnius*, while responding to the secretion, are too capricious to serve as a preparation for assay. It was found that the addition of the secretion to the exposed heart of the cockroach *Periplaneta americana* resulted in an increased rate of beating of the heart; this preparation was used extensively in the investigation. A second effect of the secretion provided information about the identity of the active principle. When injected into hypophysectomized specimens of the frog, *Rana pipiens*, the secretion causes the pigment in the melanocytes of the skin to expand slightly.

Materials and Methods

Extracts of the Glands

Because they were more readily available in quantity, the utriculi majores of *Periplaneta* were used as a source of the secretion. The cockroach was dissected and the glands removed under insect saline (11). The glands were

[1]Manuscript received September 28, 1959.
Contribution from the Department of Zoology, University of Toronto, Toronto, Ontario.
[2]National Research Council Fellow.

ground in a glass homogenizer together with either insect saline (11) or frog Ringer's solution, depending on the eventual use of the extract.

Cockroach Heart Preparation

The isolated heart of *Periplaneta* has long been used in insect pharmacology and the method of preparation has been described by Yeager (11). The isolated heart, pinned on a wax block, was suspended vertically in a tubular chamber containing insect saline which was stirred and aerated by a stream of bubbles of oxygen. The chamber could be drained through a tube at its lower end and filled from a reservoir. The experimental solutions were introduced into the chamber by means of a pipette. The rate of heart beat was observed through a dissecting microscope and was recorded as the number of beats per minute, determined by counting the beats over a 1-minute interval. Under the conditions described, the heart rate became constant within an hour of the making of the preparation and was useful for about five hours. Although the heart rate usually became constant within 5 minutes after the preparation was rinsed with fresh saline, a period of 15 minutes was normally allowed to elapse before the preparation was used again.

It must be realized that the heart rate of any individual cockroach was not necessarily the same as any other and that two different heart preparations might have different sensitivities to the same extract of cockroach accessory secretion. Because of this, it is difficult to compare the results from one heart preparation with those from another; accordingly a complete summary of the numerical results obtained would accomplish nothing and this has not been included. However, it is stressed that all of the observations reported here are based on at least 10 experiments, frequently more, and that unless it is stated otherwise, each of the trials gave the same qualitative results.

Melanocyte Preparations

Specimens of *Rana pipiens* were anaesthetized with urethane and the pituitary body was removed through a three-sided incision in the floor of the skull. Within 8 hours animals treated in this way had blanched and the pigment in the melanocytes had contracted. Observations were made photographically on an area of the web of the foot as described elsewhere (5). For the experiments involving isolated skin, a web of the foot was removed from an anaesthetized frog, the upper layer of skin was carefully separated from the lower layer, and the two sheets of skin were pinned on wax blocks under saline (9). After two or three rinsings spread over an hour, the pigment in the melanocytes was completely contracted.

Enzyme Preparations

Trypsin and tyrosinase were obtained as the crystals from Nutritional Biochemicals Corporation, Cleveland, Ohio. The monoamine oxidase was extracted from pork liver (7) and the preparation was assayed for activity in the Warburg apparatus using an excess of tryptamine as a substrate. At a pH of 7.4, the Q_{O_2} of the enzyme preparation was 17 $mm^3/ml/hr$. The *o*-diphenolase was extracted from sweet potatoes (6); using an excess of

catechol as a substrate, it had a Q_{O_2} of 26 mm³/ml/hr. Its lack of monophenolase activity was demonstrated by the lack of oxygen uptake when tyrosine was used as the substrate.

The Effect of the Secretion on Melanocytes

When extracts in Ringer's solution of the utriculi majores from a single *Periplaneta* are injected into the dorsal lymph space of a hypophysectomized frog, the pigment in the melanocytes of the foot expands slightly but significantly within 10 to 15 minutes, the degree of dispersion corresponding to a change from 0 to 1.0 in the melanophore index. In this respect the secretion resembles the indolalkylamines such as tryptamine and 5-hydroxytryptamine (5). The degree of dispersion of the pigment in response to both the secretion and the indolalkylamines is approximately the same and appears to be independent of dose when the test is carried out on hypophysectomized frogs. When isolated pieces of frog skin are used, however, preliminary experiments indicate that the degree of dispersion of the pigment is dependent on the concentration of the indolalkylamine or secretion present. In the case of the secretion, the dispersion lasts for up to 2 hours, whereas the pigment returns to its normal contracted state within an hour after the administration of an indolalkylamine.

The Action of the Secretion on the Insect Heart

When extracts in insect saline of the utriculi majores are added to the saline bathing a preparation of the heart of *Periplaneta*, the rate of beating increases slowly, reaching its maximum about five minutes after the addition of the secretion. Although there is considerable variation in the activity of extracts from various individuals, a suspension of the utriculi majores from one cockroach in 250 ml of the fluid bathing the heart is sufficient to increase the rate by about fifty per cent. The addition of extracts of other insect tissues like fat body or other accessory glands had no effect on the heart. Moreover, the increase in heart rate depended on the concentration of the secretion in the insect saline bathing the heart, as is shown by the following sample of data. The heart rate of the preparation used was 65/min before any secretion was added. After addition of a quantity equal to 1/10 of the material from one animal, the rate rose to 74. When double this amount was added the rate rose to 98/min, and when five times the original amount was added, the rate increased to 120/min. After each addition the preparation was rinsed and the rate returned to 65/min.

This ability to increase the rate of beating of the cockroach heart is a property which is shared by a large number of compounds, including the indolalkylamines. Consequently, these experiments cannot in themselves tell us very much about the identity of the active principle in the secretion; they can, however, serve as a basis for detecting the presence of the material in extracts which have been subjected to various treatments.

The Effect of Enzymes on the Secretion

The ability of the secretion to excite the cockroach heart is unaffected by heating extracts at 100° C for 10 minutes or by incubating them with trypsin at 37° C for 6 hours. The action of certain other enzymes, however, destroys the activity.

When the monoamine oxidase enzyme preparation was fresh, 0.2 ml of the enzyme preparation added to 3 ml of heart saline containing the utriculi majores from three cockroaches completely destroyed the activity of the extract in 30 minutes. In this experiment, as in the others involving enzymes, the decrease in activity was followed by adding aliquots of the suspension to the cockroach heart preparation. When the monoamine oxidase extract was less fresh, the time required for complete inactivation of a similar preparation of accessory secretion was extended to as much as 2 hours. For all of the enzyme experiments, the control consisted of an extract of the gland which was incubated with a boiled enzyme preparation. In each case the activity of the extract remained unchanged.

The enzyme tyrosinase also inactivated the secretion when 0.2 ml of a solution containing 1 mg/ml of the crystalline enzyme was incubated with 2.0 ml of heart saline containing the utriculi majores from two cockroaches. In this case, about one hour was required for the complete inactivation of the extract. In addition, the suspension took on a red color, probably as a result of the formation of pigments which are the products of tyrosinase action. The controls remained unchanged with respect to both activity and color.

This reduction in activity by tyrosinase could be the result of an oxidation of a mono- or di-phenol group. In order to discover which was involved, the enzyme *o*-diphenolase was employed; this enzyme exhibits only diphenolase activity. Although the deactivation of the extracts of the gland was not so striking as with the other enzymes, some decrease in activity did occur. In the best of the experiments, the suspension used increased the heart rate by 28% before treatment with the enzyme and by only 7% after incubation with the enzyme for 1 hour. In other trials, the activity fell to a low level, but later increased to its former level. For instance, in one experiment, the addition of an aliquot of a suspension of glands containing some of the enzyme preparation increased the heart rate by 36% at the beginning of the experiment, by 34% after 1 hour, by 16% after 2 hours, and by 30% after 3 hours. The significance of this later increase, which occurred in 6 of 11 trials, is unknown.

The Particulate Nature of the Secretion

The opaque accessory secretion from both *Periplaneta* and *Rhodnius* is made up of densely packed particles. If an extract of the glands in insect saline is centrifuged to remove all of the particles, the clear supernatant retains some of the activity of the original suspension when placed on the isolated cockroach heart. However, if the centrifugate is resuspended in fresh insect saline and then centrifuged again the supernatant again will increase the rate of beating of the heart. The following are the results from a typical

experiment of this sort. Before centrifugation, 0.5 ml of the suspension increased the heart rate by 53%. The supernatant which resulted from centrifuging the suspension increased the heart rate by 31% and the supernatant which resulted from resuspending the centrifugate and centrifuging the second suspension increased the heart rate by 26%.

The Effect of an Indolalkylamine on the Oviducts of Rhodnius

We must turn now to the function of the secretion in the insect. It will be remembered that the secretion, acting through a peripheral nervous system, causes the oviducts to contract and that this is manifested in the isolated oviducts by an increased rate of contraction after the addition of the opaque secretion (4). In view of the similarity between the secretion and the indolalkylamines, the effect of one of the latter, serotonin or 5-hydroxytryptamine, on the oviducts was of interest. Accordingly, the isolated oviducts of *Rhodnius* were exposed to various concentrations of serotonin up to 10^{-3} *M*. At no concentrations did the rate of contractions increase; in several cases it decreased.

Further research has shown that serotonin is capable of preventing or partially inhibiting the normal increase in frequency brought about by the opaque secretion. In these experiments, an oviduct was dissected from a female *Rhodnius* and pinned out under insect saline as previously described (4). After the frequency of the contractions induced by the operative procedure had fallen below one in 30 seconds, a known quantity of opaque secretion in saline (usually one-half the material from a single cockroach) was added to the preparation, resulting in an increase in frequency. This frequency was measured, and a quantity of serotonin sufficient to bring the concentration in the saline bathing the preparation to 10^{-3} *M* was added. This had no effect beyond decreasing the frequency slightly in 7 out of 10 trials. A further quantity, equal to that used initially, of the opaque secretion was added; in none of the 10 experiments was there a significant increase in the frequency of contractions. In control experiments, where no serotonin was added, the addition of the second aliquot of the extract of the gland always resulted in an additional increase in frequency. This elaborate experimental protocol is necessary to ensure that the oviduct preparation, which is capricious, is capable of responding to the secretion in the first place. In experiments where the concentration of serotonin was lowered to 10^{-5} *M* and the amount of material in the second aliquot was increased, some rise in the frequency of contractions was detected.

The following samples of the data obtained illustrate these results. In one preparation, the interval between contractions was 50 seconds before the experiment began. After the first aliquot of secretion was added, the interval fell to 25 seconds and remained at this level after the addition of sufficient serotonin to bring the concentration to 10^{-3} *M*. After the addition of a further aliquot, the interval was 23 seconds. In another experiment, the first aliquot of secretion initiated contractions in a quiescent preparation at the rate of

one every 30 seconds. When serotonin was added to bring the concentration to 10^{-5} *M*, the interval between contractions rose to 80 seconds. The addition of the second aliquot shortened the interval to 69 seconds.

Discussion

The evidence presented in this paper suggests that the active principle in the opaque accessory secretion of the male reproductive system of insects is a dihydroxyindolalkylamine. The evidence that the material is an indolalkylamine rests largely on the effect of the secretion on the melanocytes of the frog. The specificity of this reaction is therefore important. A detailed discussion of the materials which can affect the melanocytes will appear in a later communication; it is sufficient to say here that of an impressive list of compounds tried by various workers, only the indolalkylamines elicit the response described. Even compounds like tyramine and histamine, which are close to the indolalkylamines with respect to other pharmacological properties, are without effect on the melanocytes (5).

The failure of trypsin and heating to affect the material eliminates the proteins and the polypeptides from consideration. The action of monoamine oxidase confirms that an amine group is involved. The fact that tyrosinase acts on the material is a good indication that a phenol group is part of the compound. While the effects of *o*-diphenolase are not particularly striking, there can be no doubt that the enzyme acts on the material. Since this enzyme preparation exhibits only diphenolase activity, the compound must contain an *o*-diphenol group.

The ability of serotonin to inhibit the action of the secretion on the isolated oviducts of *Rhodnius* is open to a number of explanations, but in view of the other results, the following interpretation is held to be most likely. Suppose that the opaque secretion acts at certain sites on the peripheral nervous system through which it exerts its effect on the oviduct and that serotonin is sufficiently like the active material in the secretion to occupy these sites, but is not sufficiently similar to evoke a response from the nerve. Under these conditions, an inhibition of the action of the secretion by serotonin would occur.

The active material in the secretion resembles the serotonin of the mammalian gut in that it is associated with particles from which it is released into solution (1). This slow release of the material from the particles, where it is presumably bound in an inactive form, provides a possible explanation for the greater duration of the dispersion of the pigment in the frog melanocyte and for the slowness with which the heart rate of *Periplaneta* increases after the addition of the material. A further correspondence between the particles which make up the secretion and the enterochromaffin granules which are the source of the serotonin in the mammalian gut may be found in their histochemical properties. Preliminary studies along these lines have revealed that like the enterochromaffin granules, the secretion particles are argentaffin and chromaffin.

There is, then, in the male reproductive system a pharmacologically active compound, probably an *o*-dihydroxyindolalkylamine, which is bound to particles which make up the opaque accessory secretion. When placed into the female reproductive system these particles release the compound slowly, causing the oviduct to contract rhythmically. The existence of a pharmacologically potent compound requires that there be some mechanism for inactivating it. This study has revealed that at least two enzymes, monoamine oxidase and tyrosinase, are capable of rapid breakdown of the compound. The distribution of the former among insects is unknown, but the presence of the latter is well known.

Whether or not similar compounds will be found to be involved in other aspects of insect neurophysiology remains to be seen, but the possibility that an indolalkylamine may be involved in neuromuscular transmission deserves consideration, especially in view of the fact that acetylcholine does not appear to fulfill this function (10). Further, Cameron (2) has identified the pharmacologically active secretion of the corpus cardiacum of insects as an *o*-diphenol, but on the basis of his tests, the material could easily be an *o*-dihydroxyindolalkylamine. The fact that Carlisle (3) has tentatively identified the neurohomor of the crustacean pericardial cells as 5, 6-dihydroxytryptamine lends some weight to such a possibility.

Acknowledgments

I am indebted to Dr. W. E. Beckel, in whose laboratory this work is being done, for his interest and enthusiasm and for reading the manuscript, to Dr. E. H. Colhoun of the Science Service Laboratory, London, Ontario, for advice on several matters, and to the National Research Council for their generous financial support.

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FURTHER OBSERVATIONS ON THE MORPHOLOGY OF DITYLENCHUS DESTRUCTOR THORNE, 1945 (NEMATODA:TYLENCHIDAE)[1]

L. Y. Wu

Abstract

Further studies of *Ditylenchus destructor* Thorne, 1945, showed that the lips frequently have very fine annules. The lateral field of the body wall usually had 6 incisures but the number varied from 6 to 11. The cervical papillae appeared to lie in the region between the median bulb and the esophagointestinal junction between the two inner incisures. The excretory system had a long, sclerotized, terminal duct and a single lateral canal usually on the right side.

Ditylenchus destructor was described and illustrated by Thorne in 1945 (2); its morphology was studied by Wu in 1958 (3), with particular attention to the reproductive systems and the esophageal glands. This is a report on morphological details of the lips, cuticular structures, and excretory system observed in nematodes found on various host plants raised in a greenhouse. Studies were made with living specimens placed on glass slides under cover glasses with a minimum amount of water. Preserved specimens were also used for study of the lips.

Of the six lips two are lateral and two pairs are sublateral. Although apparently smooth, under high magnification a few very fine annules are often visible. Amphid apertures are well shown *en face* (Fig. 1) as two small dots.

There are six incisures on each lateral field. Two incisures are seen about two spear lengths from the anterior end, four immediately in front of the median bulb, and six about midway between the median bulb and the esophagointestinal junction. These six incisures extend backward more or less equally spaced. In some cases the region between the two inner incisures is slightly wider than between the others.

In the male the six incisures extend into the bursa. The most ventral incisure disappears as it enters the bursa (Fig. 2), while the remaining five disappear in a ventrodorsal order, the most dorsal of the incisures extending to a point just anterior to the end of the bursa.

In the female the six incisures extend caudad past the anal area (Fig. 3). Each of the two innermost incisures become wavy and unite with the adjacent outer incisure shortly behind the anal area. The resulting four incisures continue backward to about the middle of the tail, the dorsal and ventral incisures curving away from the others a short distance before they disappear.

In some cases the joining of the incisures is different. Sometimes the two dorsal incisures unite, as also do the two ventral incisures, about the middle of the tail; the remaining two incisures extend along two-thirds of the tail. Not infrequently as many as 5 additional incisures have been observed,

[1]Manuscript received September 24, 1959.
Contribution from the Nematology Section, Entomology Research Institute, Canada Department of Agriculture, Ottawa.

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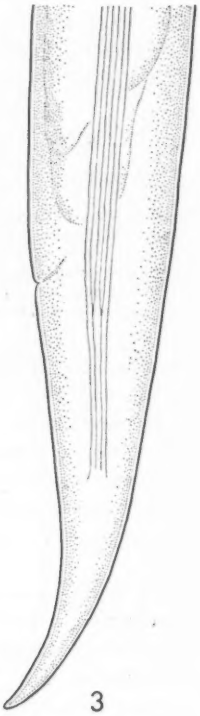

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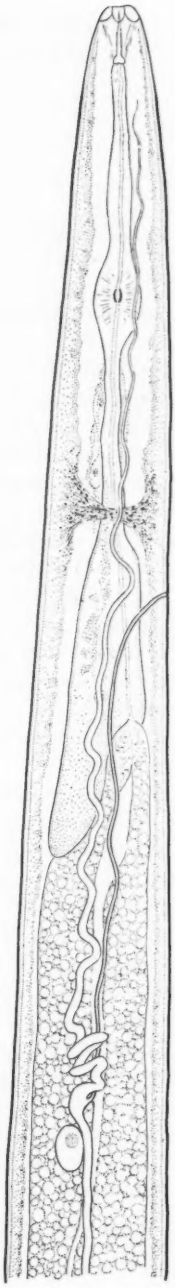

5

arising between the other 6 to form a total of 11. However, sometimes fewer than 5 additional incisures are present and the number may vary from 6 to 11.

The cervical papilla appears to be represented by a small, dotlike structure (Fig. 4) about midway between the median bulb and esophagointestinal junction just anterior to where the six incisures appear.

The excretory system consists of a terminal duct and a single canal running from near the base of the spear to the tail region.

The terminal duct is very long, being over two-thirds of the length of the esophagus. Its wall is sclerotized.

The excretory canal usually lies on the right side of the body cavity, sometimes on the left. At the anterior end it starts as a very fine tube which gradually increases in diameter and becomes more irregularly wavy in outline as it proceeds caudad. Just anterior to its junction with the terminal duct, it is a widely dilated tube which shows a few spiral turns and then enlarges further to form an ampullalike structure, the excretory sinus. A large nucleus is located usually immediately behind the excretory sinus but occasionally some distance behind it. From the excretory sinus the canal continues caudad as a fairly large and more or less straight tube. About halfway down the body this canal gradually decreases in diameter, becoming much smaller near the vulvar region where it forms some loops. From there it continues cauded as a narrow tube which can be traced into the tail for about three-quarters of the tail length.

Discussion

One of the taxonomic characters of the genus *Ditylenchus* has been that the lips of these nematodes are smooth. In the present study a few extremely fine annules were very often observed on the lips. It may be because of their fine nature that these annules have been overlooked.

The small nucleus that was reported (1) as present in the wall of the terminal duct of *Ditylenchus* was not observed during the present study.

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Figs. 1–5. *Ditylenchus destructor*. 1. Lips, *en face*. 2. Male tail. 3. Female tail. 4. Cervical papilla between four lateral incisures. 5. Anterior end of female, showing excretory system.

THE TIMING OF THE ACTIVITY PATTERN OF PEROMYSCUS IN CONSTANT DARKNESS[1]

R. H. STINSON

Abstract

The timing of the activity period of 12 adult and 2 young deer mice (*Peromyscus*) was studied in constant darkness. With both of the young and nine of the adults the activity began earlier each day. The rate of the shift varied between animals, being highest in one of the young (56 minutes per day); the higher rates appeared to be linear. The imposition of alternating light and dark prevented the shift, and constant light reversed it.

Introduction

It has been shown by Johnson (4) for *Peromyscus* and by Brown, Shriner, and Ralph (2) for the laboratory rat that when these mammals are placed in constant light the time of occurrence of the major portion of daily activity is delayed, with the amount of delay a function of light intensity. When placed in constant darkness on the other hand, the pattern does not shift; the timing remains close to that present when the animal entered the dark, and Johnson stated that no procedure had been found which would make the "clock" run fast. However, a forward shift in constant darkness has now been found by Rawson (7) in *Peromyscus leucopus* in activity wheels, and by Wolf (9) in the Japanese dancing mouse, Calhoun (3) in a single *Sigmodon*, and Aschoff in the house mouse (1). In the present study the activity patterns of two other forms of *Peromyscus* in addition to *P. leucopus* were examined in constant darkness and in a type of activity cage in which general movement rather than running activity was recorded.

Methods and Materials

The three forms of deer mouse studied were *Peromyscus leucopus noveboracensis* trapped in nearby wood lots, *P. maniculatus gracilis* from Algonquin Park, Ont., and *P. maniculatus bairdii* bred in the laboratory from a stock trapped at Long Point, Lake Erie. Those of the *leucopus* and *bairdii* types were 3 years old, the *gracilis* 4 years old. Previous to the experiment they had been kept in a former dark room and had lived for several months in a condition of semidarkness. Whether any relationship existed between the mice was not known.

The activity pattern was examined by means of a tilting cage into which an animal entered from a nest box (5). The movements of the cage closed and opened an electrical contact which actuated a pen of an Esterline-Angus operation recorder. Two light-tight chambers mounted in a dark room held six activity cages. In one chamber six *P. leucopus* were placed, in the other,

[1]Manuscript received June 17, 1959.
Contribution from the Department of Zoology, University of Western Ontario, London, Ontario. This research was carried out under a National Research Council Summer Research Associateship, 1956.

three of *gracilis* and three of *bairdii*. Each cage held sufficient food (fox chow) and water that the chambers could be closed for a week at a time. At the end of 3 weeks the relative positions of the cages were changed within each box in order to determine whether the activity pattern of a mouse could be influenced by that of its neighbor. The temperature of each box was $21 \pm 1°$ C; the humidity was not controlled.

The record paper moved too slowly (3/4 in. per hr) to separate individual cage movements so the activity was recorded in time units, viz., the number of 4-minute periods in which any activity took place during each 2-hour interval. Although this method of measuring activity does not give an exact count of cage movements, it does define the definite major activity period. This extended over 12 to 14 hours with the activity rising to a high level within the first 2 hours and tapering off in a similar length of time at the end of the period. There was not a precise beginning or ending to the activity and hence the times of starting and stopping could not be used as reference points of the distribution. Instead the method of Johnson (4) was used; the reference point was taken to be the weighted mid-point of the distribution, i.e., the time by which 50% of the activity (in 4-minute periods) had occurred. A weekly average was then determined for the time of the mid-point.

Results

Nine of the mice gave some evidence of a forward shift in the activity period (Fig. 1) while three of the *leucopus* showed little if any shift. The rate of shifting was quite variable (Table I). In certain mice it began within the first 2 weeks, in others a longer period was necessary before it became apparent.

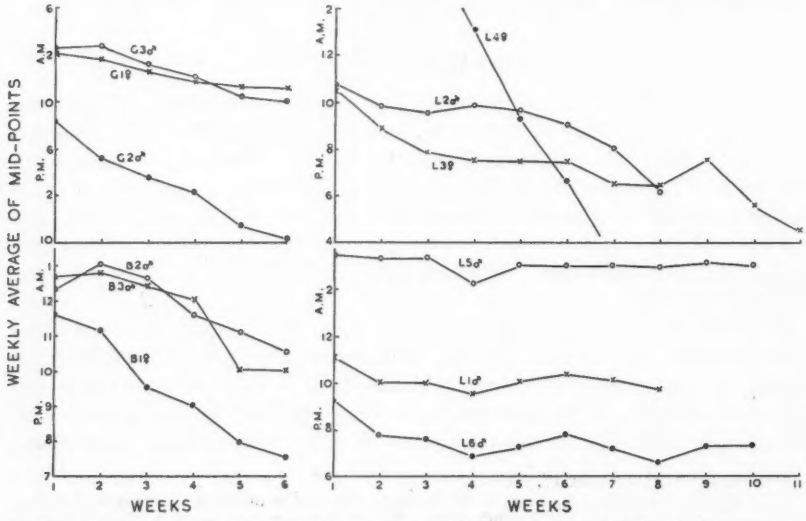

FIG. 1. Variation in the time of occurrence of daily activity periods in 12 *Peromyscus* in continual darkness.

TABLE I

Rate of forward shift of activity mid-point in 12 *Peromyscus*

Mouse No.	L1	L2	L3	L4	L5	L6	G1	G2	G3	B1	B2	B3
Rate in minutes per day	1.1	4.9	4.2	28.0	0.4	1.1	5.1	16.5	8.3	7.5	5.1	4.4

There was no evidence that the activity pattern of one mouse was affecting those of its neighbors; during the first 3 weeks L4, which shifted rapidly, was placed beside LI and during the remainder of the experiment between L5 and L6.

In those mice with the highest rates, the shifting exhibited a linearity over a period of time (Fig. 2) similar to that of the backward shift in constant light (4). The fact that on certain days the shift was not forward but backward may be due in part to lack of precise determination of the activity mid-point, but it is evident in the daily records of all the mice and illustrates a tendency for the shift to proceed by jumps every few days.

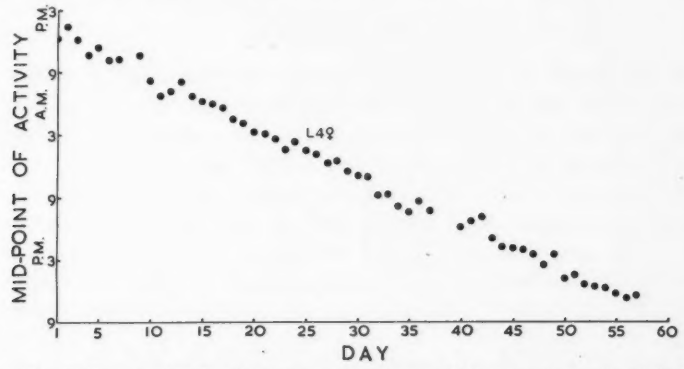

FIG. 2. Daily rate of shift of activity pattern in one *P. leucopus* in continual darkness.

Two effects of light upon the shift are shown in Fig. 3. In (*a*) is the record of a young *leucopus*, 2 months old, which had been born and raised in the darkroom. When first tested, its activity shifted forward at a rate of 48 minutes a day. A litter mate possessed a forward rate of 56 minutes a day. After 5 weeks in the dark L7 was subjected to a 12-hour light–dark regime in order to determine whether the rapid forward shift could be stopped. When returned to the constant darkness the shift proceeded at a lower rate.

In Fig. 3 (*b*) is an example of a complete reversal of the shift under constant light. Johnson (4) used a light intensity of 24 ft-c while Rawson (7) needed only 0.34 ft-c to obtain a delay of about 90 minutes a day; here a similar rate was achieved in L2 with an intensity of only 0.09 ft-c. It is likely that considerable variation exists between animals in the rate of the backward as well as the forward shift. Brown, Shriner, and Ralph (2) recorded a delay in the activity cycle of the rat of 1.25 hours a day under a constant illumination of 1 ft-c.

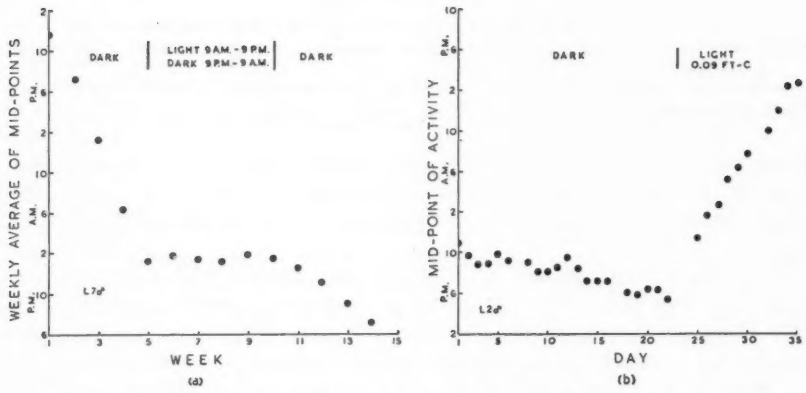

FIG. 3. Effects of light upon the forward shift of the activity period of two *P. leucopus*.

Discussion

These results confirm those of Rawson (7) and Pittendrigh and Bruce (6) that there is a forward shift or a decrease in the period of the rhythm in species of *Peromyscus* when held in constant darkness. They also support the concept of the endogenous nature of the rhythm. Although Webb and Brown (8) state there is evidence that rhythms in organisms may be controlled by fluctuations in environmental factors such as air pressure and high energy radiation, the fact that neighboring animals in activity cages may have rhythms of different frequencies and at times be completely out of phase with one another suggests that in *Peromyscus* each animal has its own endogenous control.

The use of the relaxation oscillator as a model in attempting to explain the cyclic nature of activity rhythms has been discussed by Pittendrigh and Bruce (6). The frequency of such an oscillator can be varied in different ways, for example by altering the time constants of circuits or by changing the input, and it can be made to retain a more or less constant frequency under any one set of conditions. Following the oscillator analogy with *Peromyscus*, the linear nature of the forward drift in certain animals (Fig. 2) and that of the backward drift shown also by Johnson (4) suggests that as in an oscillator a relatively simple and sometimes rapid switch (Fig. 3) may be made and held at a rather constant frequency under a new condition. Since the rate of backward drift is a function of light intensity (4) there is apparently from Fig. 3(*b*) a low light intensity which when presented continuously, or possibly at some interval, would maintain a rhythm with the period of a solar day. How light, or the lack of it, alters the characteristics of the animal "oscillator" in such a way as to bring about the above frequency changes is not known.

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AN INSECT HOST–PARASITE POPULATION[1]

THOMAS BURNETT

Abstract

Eight populations of *Trialeurodes vaporariorum* and *Encarsia formosa* were propagated on tomato plants in the greenhouse for about eight months. Although there was some variation in the growth-forms of host and parasite among the experiments, the general trend in each experiment was one of fluctuations of slightly, but distinctly, increasing amplitude. As extraneous sources of variation had only a slight influence on the growth-forms, the fluctuations resulted from host–parasite interaction. The *T. vaporariorum* – *E. formosa* system is well suited for an investigation of the principles of host–parasite interaction.

When the numbers of an appropriate stage of an insect species are recorded at a given period each year it is found that the increase or decrease in the size of the population from one year to the next is limited, often by a factor of five or less. It is also found that the usual fluctuations in numbers lie between limits which appear to be restricted in comparison with the variation which the remarkable powers of increase possessed by most insects would permit. The restricted variation in population density suggests that population growth is controlled in some manner. As the density of a species is not correlated with its intrinsic rate of increase, attention has centered on the very many agencies which cause mortality throughout the life cycle of insects. Although the significance of these mortality factors in the determination of insect numbers is a matter of great controversy the role of that unique predator, the insect parasite, is usually considered to be one of potentially great importance for a number of reasons: parasites have the ability, over a number of generations, to increase the percentage of hosts killed with increasing host density and to decrease the percentage of hosts killed with decreasing host density; the successful results of the biological method of pest control indicate the ability of insect parasites to reduce and limit population densities in some cases; and various mathematical theories have predicted the regulation of the densities of host and parasite species through the processes of host–parasite interaction. As part of an investigation of the abundance of insect species it is necessary, therefore, to determine both the relationship between parasites and their host populations and the influence of the physical and remaining biotic environment on this relationship.

One useful approach to the problem of host–parasite interaction is to remove the species from the natural community and rear them under controlled conditions. Because this eliminates most of the variables present in nature the results of the simplified system will not be directly comparable with natural events; but the controlled investigation permits an analysis of the biological factors involved in this particular interaction, and additional variables may be

[1]Manuscript received October 22, 1959.
Contribution from Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario.

added, one at a time. When the generations of host and parasite are discrete —as in species with one generation per year—a model of host–parasite interaction may be constructed by arbitrarily limiting the rate of increase of host and parasite populations. By this means DeBach and Smith (5) followed the growth-forms of *Musca domestica* L. and its parasite *Nasonia vitripennis* (Walk.) for seven generations. In a similar experiment covering 21 generations the numbers of *Trialeurodes vaporariorum* (Westw.) and its chalcid parasite *Encarsia formosa* Gahan passed through two fluctuations of increasing amplitude (3).

In many instances, however, the generations of host and parasite are not discrete and to observe the consequences of host–parasite interaction in these circumstances it is necessary to propagate freely interacting populations of the two species. Flanders (6, 7) established a self-perpetuating system using the flour moth *Anagasta kuhniella* (Zeller) and the wasp *Exidechthis canescens* (Grav.). The hosts were reared on rolled wheat in a number of small stender jars. As the parasite's ovipositor could penetrate only 6 mm into the food, the depth of the food and the movement of the host larvae throughout it were factors of primary importance in determining the success of the parasite population in finding hosts. Utida (see 12 for references) in a long series of papers analyzed the growth-forms of combinations of the azuki bean weevil, *Callosobruchus chinensis* (L.), and the cowpea weevil, *Callosobruchus maculatus* (Fab.), with the parasites *Neocatolaccus mamezophagus* Ishii and Nagasawa and *Heterospilus prosopidis* Vier. when these insects were reared on beans in Petri dishes. In many of the experiments host density was sufficiently high at times to be a factor in reducing its reproductive rate so that the oscillations of both host and parasite populations were damped. Utida (11) quoted Takahasi's results with experimental populations of the flour moth *Xenephestia cautella* (Wlkr.) and its larval parasites *Bracon hebetor* Say and *Cimodus* sp. In studies related to host–parasite interaction the growth-forms of acarine predator–prey populations have been recorded and analyzed: Huffaker and Kennett (8) investigated the interaction of *Tarsonemus pallidus* Banks and its predator *Typhlodromus* sp. on strawberry plants reared in the greenhouse and later Huffaker (9) recorded three oscillations of the mite *Eotetranychus sexmaculatus* (Riley) and its predator *Typhlodromus occidentalis* Nesbitt in laboratory cultures; and Collyer (4) tested the ability of some predaceous mites to regulate the density of *Panonychus ulmi* (Koch) populations when this prey was reared on *Prunus insititia* L. in the greenhouse.

Previous experiments in which *Trialeurodes vaporariorum* and *Encarsia formosa* were reared on four tomato plants in the greenhouse indicate that this experimental population has many advantages for a study of host–parasite interaction (2). All stages of both species are found on the underside of the leaf of the host plant and with the exception of the first larval instar of the host, which moves very small distances, only the adults of both species are not sedentary. The susceptible stages of the host—the second, third, and early fourth larval instars—are exposed to attack by the parthenogenetic parasite at all times. Parasitized hosts become blackened in a few days while

unparasitized hosts remain white. As adult male and female hosts are attracted to succulent foliage the host population, followed by that of the parasite, gradually moves up the host plant. Thus the populations of both species are distributed, according to age, in a series of planes. The quantity and quality of host plants may be varied as desired. The influence of physical factors on this host–parasite system is very great; for example, the ability of the parasite to limit host increase is closely dependent on temperature. It is probable that host and parasite populations increase equally at a temperature slightly below 24° C. Below this temperature the host population is not limited by the parasite while above it the parasite becomes increasingly dominant with increasing temperature. The populations of both host and parasite can be counted and their distribution recorded without disturbing the mobile stages of either species.

The experiments mentioned above indicate that *T. vaporariorum* – *E. formosa* populations can coexist and fluctuate for at least five months in an environment as small as four tomato plants. Two factors, other than variation in host and parasite density, appear to have influenced the results obtained in these experiments. First, each experiment was begun by placing one lot of adult hosts and, later, one lot of adult parasites on the host plants. Under these circumstances life-history phenomena initiated the fluctuations in the numbers of each stage of each species. Second, periods of high mortality of second and third stage host larvae (which produced neither adult hosts nor parasites) occurred only when a large number of adult parasites were present. This biological factor may be critical for the coexistence of the two species in a limited environment as it results in an immediate reduction in the number of adult parasites and a subsequent higher survival of host larvae.

The importance of these two factors, along with those of host and parasite density, in determining the growth-forms of *T. vaporariorum* and *E. formosa* populations may be estimated by infesting many host plants gradually and rearing the populations as long as possible. This procedure allows a comparison to be made of both the parasitism and host mortality within leaves, between leaves of the same plant, between plants, and between groups of plants.

The necessity of using many host plants requires large experimental areas such as sections of a greenhouse. The physical environment is not entirely uniform in this type of experimental unit because of seasonal change and variation from one area to another at any given period. In addition, the quality of the host plants may vary subtly with the season. As the response and metabolic physiology of both host and parasite species are affected by variation in their physical and biotic environments (1, 2) it is necessary to know if the variations in the numbers of host and parasite result primarily from host–parasite interaction before the factors of the interaction itself can be analyzed.

This paper deals with preliminary experiments on the growth-forms of *T. vaporariorum* and *E. formosa* populations propagated in the greenhouse.

Materials and Methods

All the stages of the greenhouse whitefly, *Trialeurodes vaporariorum* (Family Aleurodidae, Order Homoptera), occur on the underside of the leaf of the host plant. The elongate-oval eggs are stalked. The other immature stages are generally referred to as the four larval and one pupal instars. The first larval instar is active at first and wanders over the undersurface of the leaf. During the latter part of this stadium the legs become rudimentary and thereafter this and the remaining immature stages are quiescent. The second and third instars are flat and are surrounded by a thin marginal fringe of wax. During the fourth stadium the body is thickened and is elevated on a palisade of wax rods. The formation of the so-called pupa takes place within the last larval skin, the puparium, and the adult emerges through a T-shaped opening in the dorsal wall of the puparium. Both males and females are produced. At 24° C the average time of development is almost 25 days, and the median longevity of the adult females is about 17 days in the laboratory and about 12 days in the greenhouse (2, Tables VII, X, and XII).

Encarsia formosa (Family Eulophidae, Order Hymenoptera) is an internal parasite of *T. vaporariorum*. The female lays its eggs through the dorsal surface of the quiescent larval instars of the host. The larva appears to have three instars (10). When the inside of the host larva has been consumed by the parasite larva, the shell of the host turns black. The parasite larva pupates inside the shell of the host, and the adult (approximately one mm long) emerges through a hole cut in the dorsal wall. The adults are usually all females but occasionally a small number of males appear. At 24° C the average time of development is 15 days and the median longevity of the adult is about 16 days in the laboratory and 3–4 days in the greenhouse (2, Tables VII, X, and XII).

The host plant used in the experiments was the tomato, *Lycopersicon esculentum* Mill. variety Camdown.

Eight populations of *T. vaporariorum* and *E. formosa* were each reared in a 14- by 17-ft section of a greenhouse which was shaded as required. Four populations, called A, B, C, and D, were propagated simultaneously and the following year a second set of four populations, E, F, G, and H, were reared in four adjoining greenhouse sections. The sections containing populations A and B were connected by removing three panes of glass, each 15 by 20 in., from the partition separating the two sections, and three panes were also removed from the partition separating populations C and D. The sections containing the remaining four populations were isolated from each other. Host plants were introduced into the experimental units when they were 2 months old and had five to six leaves. After 1 month in the sections the plants had 10–12 leaves and any further growth, either terminal or lateral, was removed. At the end of the second month the plants were replaced by new ones and placed under the greenhouse benches for 2 weeks to permit any parasites they contained to emerge within the experimental area. The

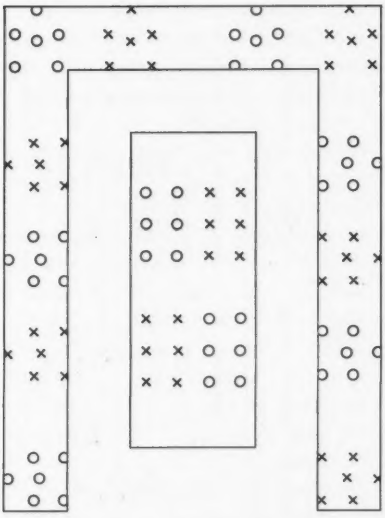


FIG. 1. Distribution of host plants in experimental unit. Circles: tomato plants of one age group. Crosses: tomato plants 1 month older than first age group.

temperature in each section was usually maintained at 23° C (with a range up to 27° C); on a few occasions it rose to about 27° C or fell below 23° C for a few hours.

Seven populations, A–G, were reared on 96 tomato plants, half of the plants being replaced every month. The two age groups of plants were arranged on the north, west, south, and center benches of the greenhouse sections in alternate blocks of six as indicated in Fig. 1. The eighth population, H, was reared on 64 tomato plants, one quarter of the host plants being replaced every half-month. The four age groups of plants were arranged on the benches in 16 identical blocks of four plants each; each block contained one plant belonging to each age group.

Each experiment was begun by adding enough adult parasites to a gradually increasing host population to cause a subsequent gradual decrease in the numbers of both species. As the numbers of each species necessary for limited initial fluctuations were not known the infestation schedules of *T. vaporariorum* and *E. formosa* were varied according to the estimated (by observation) rates of increase of immature and adult host and parasite populations. Populations A, B, C, and D were started by keeping a heavily infested plant, containing all stages of host and parasite, in each section for 13 days with the 48 tomato plants there at that time. Leaves heavily infested with parasitized host scales were kept in each section from the 14th to the 24th day of the experiments. In addition, 100 adult parasites were introduced into each population on each of 4 days, the 18th, 19th, 20th, and 21st day of the experiments. The first census of these experiments was made on the

TABLE I

Infestation schedule

Population E					Population F					Population G					Population H				
	Number of hosts		Number of parasites			Number of hosts		Number of parasites			Number of hosts		Number of parasites			Number of hosts		Number of parasites	
Day	Added	Present	Added	Present	Day	Added	Present	Added	Present	Day	Added	Present	Added	Present	Day	Added	Present	Added	Present
1	151	400	0	9	1	270	400	0	7	1	600	600	0	0	1	61	200	0	7
4	152	600	0	18	4	142	600	0	13	6	392	1000	0	8	4	145	400	0	13
8	70	1000	0	62	8	242	1000	0	68	9	519	1500	0	23	8	137	600	0	79
12	0	1900	0	285	12	479	1500	0	160	14	911	2200	0	30	11	0	1004	0	169
16	3	2200	0	834	16	303	2200	0	504	20	—	1552		31	17	0	1150	0	299
23	558	2500	0	841	23	570	2500	0	657	21	—	—	400	—	25	259	1200	0	497
30	889	2500	0	1169	30	1028	2500	0	957	23	—	—	300	—					
36	1000	—	—	—	36	1000	—	—	—	29	—	—	400	—					
										36	—	—	500	—					

60th day. The propagation of populations A, B, C, and D was begun on August 24 and ended April 1. The programs of infestation for populations E, F, G, and H are shown in Table I where the numbers of adult hosts and parasites which were present and which were added to the four populations during the first month of the experiments are recorded. Populations E and F were planned as duplicates, but it soon became evident that the larval host populations were quite different in the two sections; therefore, six tomato plants were exchanged between the two experiments. Population E was started on August 19, ended on March 28, and the first census was made on the 37th day; population F was started on August 19, ended on May 1, and the first census was made on the 37th day; population G was started on September 11, ended on March 24, and the first census was made on the 41st day; and population H was started on August 19, ended on May 1, and the first census was made on the 35th day.

The numbers of adult hosts and parasites on 16 tomato plants were counted in each population twice weekly. In populations A–G one plant of each group of six was used in each census so that eight older and eight younger plants were included. From each group of six plants, a different one was chosen for six consecutive census periods; therefore, all the plants in these seven populations were examined within a 3-week period. In population H one plant of each group of four was used in each census. The plants were chosen so that four belonging to each of the four age-groups were included in each census. From each group of four plants a different one was chosen for four consecutive census periods; therefore, all the plants in this population were examined within a 2-week period.

Species other than *T. vaporariorum* and *E. formosa* found on the tomato plants were removed.

Results

The numbers of adult *T. vaporariorum* and *E. formosa* counted twice a week on 16 tomato plants are shown in Fig. 2 for populations A and B, in Fig. 3 for populations C and D, in Fig. 4 for populations E and F, and in Fig. 5 for populations G and H. The sampling counts of populations A, B, C, and D began when the numbers of the host adults were estimated by observation to be at or near the maximum of their first fluctuation; the sampling of the remaining four populations began before the maximum numbers of the first fluctuation occurred.

The growth-forms of host and parasite adults were similar in populations A, B, C, D, and E. In populations A, B, C, and D the first fluctuation of host numbers was completed by the fourth week, the second fluctuation occurred from the fourth to the 16th week, and the third fluctuation lasted from the 16th week until the end of the experiments on the 24th week. As the census of population E began 4 weeks earlier than the other four populations the first fluctuation was not completed until the eighth week. The second fluctuation is considered tentatively to have contained two peaks of

host numbers, the second one varying in magnitude in relation to the first. As the experiments had to be discontinued after the 24th week it is not known if the third fluctuation would also contain two peaks. In the fluctuations in host numbers of these five populations the minimum numbers of adult hosts after the second fluctuation (week 18 or, for population E, week 23) was either greater (populations A, B, E) or about equal (populations C, D) to the minimum occurring after the first fluctuation (week 4 or, for population E, week 8); it was never less. As the peaks of the three fluctuations were successively greater, both absolutely and in relation to increase from the previous minimum, the average density of the host population was increasing

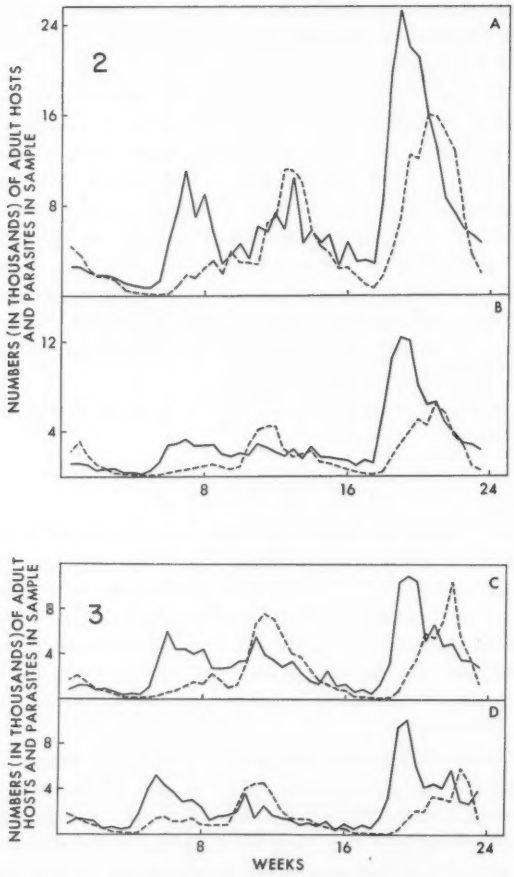


FIG. 2. Numbers of adult hosts and parasites counted in populations A and B. A, population A. B, population B. Solid line, hosts. Dotted line, parasites.

FIG. 3. Numbers of adult hosts and parasites counted in populations C and D. C, population C. D, population D. Solid line, hosts. Dotted line, parasites.

rapidly. In all five populations the variations in parasite numbers was more regular than those of the host. The second peak of parasite numbers (weeks 11–12 for populations A, B, C, D; weeks 16–17 for population E) was always greater than the first peak (weeks 0–1 for populations A, B, C, D; weeks 4–5 for population E) but in contrast to the host, the minimum of each second fluctuation was as low as that of the first. Note that although there were generally two distinct peaks in the second fluctuation of host numbers, there was only one in that of the parasite. Each increase in parasite numbers followed, with a lag, an increase in host numbers.

In contrast to populations A, B, C, D, and E, populations F and H fluctuated around a much lower average density. The first fluctuation of the host had

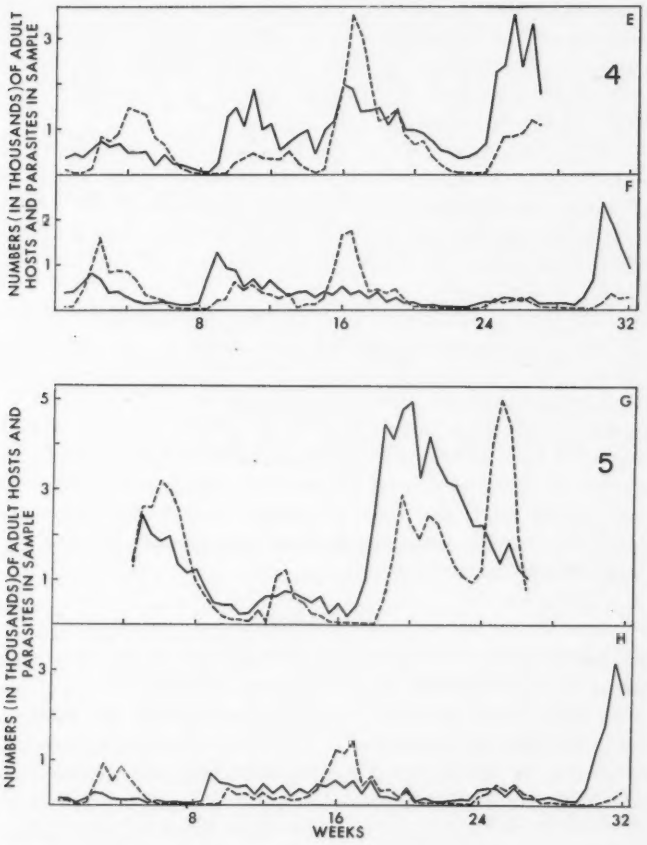


FIG. 4. Numbers of adult hosts and parasites counted in populations E and F. E, population E. F, population F. Solid line, hosts. Dotted line, parasites.

FIG. 5. Numbers of adult hosts and parasites counted in populations G and H. G, population G. H, population H. Solid line, hosts. Dotted line, parasites.

the same general pattern in all seven experiments. In the second fluctuation in populations F and H, however, a distinct second peak did not occur (as in populations A, B, C, D, and E) but instead there was a gradual decrease in the numbers of *T. vaporariorum* until the 24th week. At this time there was a slight increase and decrease in host numbers followed by a distinct third fluctuation, occurring from the 29th to 32nd weeks. The minimum numbers of hosts after the first and second peaks remained about equal but the second minimum was extended so that the third fluctuation began about five weeks later in populations F and H than in population E. Again, with the exception of the minor fluctuation at the 24th week, the parasite population tended to follow, with a lag, the variations in numbers of the host. In population F, however, the second peak was not distinctly greater than the first.

In population G the first peaks of host and parasite numbers were followed by slight simultaneous fluctuations in numbers of host and parasite (seventh to 12th weeks). This minor fluctuation was followed by the second major increase and decrease of host and then of parasite populations from the 13th to 21st weeks of the experiment.

The variations in the densities of the host larvae were not recorded in this preliminary set of experiments but it was observed that many immature hosts were killed—producing neither hosts nor parasites—in some experiments when the parasite numbers were at the maximum of a fluctuation. It was also observed that males of *E. formosa* appeared in small numbers only at the peaks of parasite emergence.

The results of the eight experiments show that populations of *T. vaporariorum* and its parasite *E. formosa* fluctuated with peaks of increasing amplitude, that the minima of host numbers were either subequal or successively higher, and that minima of parasite numbers remained low. During the 8 months of propagation there were three peaks in the numbers of the host and two (there not being sufficient time for a third peak) of the parasite. Although the amplitude of the fluctuations of the host varied a great deal from one experiment to the next, the time at which these fluctuations occurred is found to be very similar when appropriate portions of the growth-forms of any two experiments are compared.

Discussion

As the greenhouse environment is not completely uniform in its physical and biotic aspects it is possible that the pattern of variation in the numbers of host and parasite adults could be determined or greatly modified by factors other than host–parasite interaction. In this connection the influence of the following sources of variation on the growth-forms of *T. vaporariorum* and *E. formosa* populations are discussed.

Season

As the experiments were conducted for 8 months beginning in the autumn the amount and intensity of light falling on the host plants varied with the season. It is possible, therefore, that the properties of the host plants, the

host insects, and parasites were modified in a systematic manner and therefore the fluctuations of host and parasite populations could have resulted from a seasonal cycle in the physical environment. Seasonal effects on the succulence and nutrition of the host plants were reduced by growing the plants for 2 months under supplementary lighting before use in the experimental units; by fertilizing the plants during the 2 months they were used for insect propagation; by shading the experimental units as required, and by replacing the plants every 2 months. The survival of host adults after the initial infestations of population G (Table I) and the subsequent rapid increase in the numbers of host and parasite (Fig. 5, weeks 4–6) give an indication that during the period of census the mortality of the host was low and the power of increase of the host was very high.

If the fluctuations of the host and parasite populations were determined by seasonal agencies the increase and decrease in the numbers of both species should be synchronized throughout the experiments. Population G was started in an experimental unit contiguous to those containing populations F and H about three weeks after the beginning of the latter experiments. The beginning of population G was delayed so that the first peaks in the numbers of hosts and parasites would occur when the numbers in populations F and H had passed their first maximum and were approaching minimal values of the first fluctuation. Figures 4 and 5 show that although the magnitude of the fluctuations of population G varied from those of populations F and H (probably because of the initial differences between the experiments) the fluctuations of the former population remained out of phase with those of the latter populations.

Although the rates of increase of host and parasite populations may have varied slightly with the season they were always very high and the fluctuations within each experiment in the numbers of adult hosts and parasites were independent of any systematic variation in physcial factors during the period of census.

Area

In the large experimental units employed, physical factors such as temperature, humidity, and light probably vary horizontally and vertically. Temperature could have been stabilized by circulating the air in each section of the greenhouse but it was considered very important in these preliminary experiments to avoid the risk of interfering with the natural dispersal of host and parasite adults. If the physical environment were significantly heterogeneous the numbers of adults in different areas of the experimental sections would be greatly different. To investigate host and parasite distribution in the experiments each set of 16 sampling records of populations E, F, G, and H was divided into four lots representing the north, west, south, and center benches in the greenhouse section. A second test for heterogeneity was made by comparing the distribution of adult hosts and parasites on the inside and outside rows of host plants located on the north, west, and south benches. It is possible to make this comparison because the numbers of

insects were counted in the first census of each week on 12 plants of the outside row in the three areas and on the alternate census on 12 plants of the inside row.

The distributions of *T. vaporariorum* and *E. formosa* adults were uniform at moderate and high densities in all parts of the environment but at low densities some differences occurred. At moderate and high densities the peaks of host and parasite densities occurred as much as 10 days apart in different areas but the order in which the peaks occurred did not follow any consistent pattern. The greatest variability which occurred in the four populations is shown in Figs. 6 and 7 where the fluctuations of host and parasite in four areas of population F are compared. Figures 6 and 7 show that although there was considerable variability in the magnitude of the fluctuations in the four areas the parasite was associated with the host throughout the experimental area; the host did not have an area where it was shielded from attack by the parasite. The only consistent and relatively large difference in the distribution of host adults in the four areas occurred between the north and south benches from the 10th to 13th weeks; the unusually high fluctuation on the north bench was associated with and probably caused by the unusually high initial peak in host numbers. The distribution of the

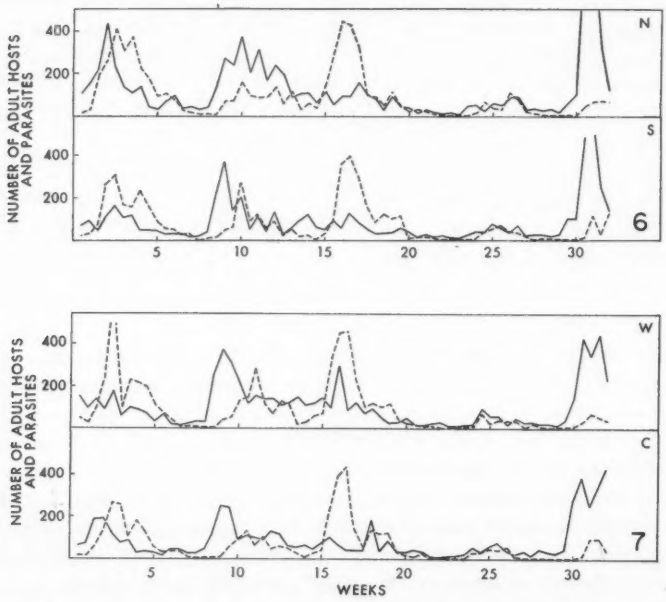
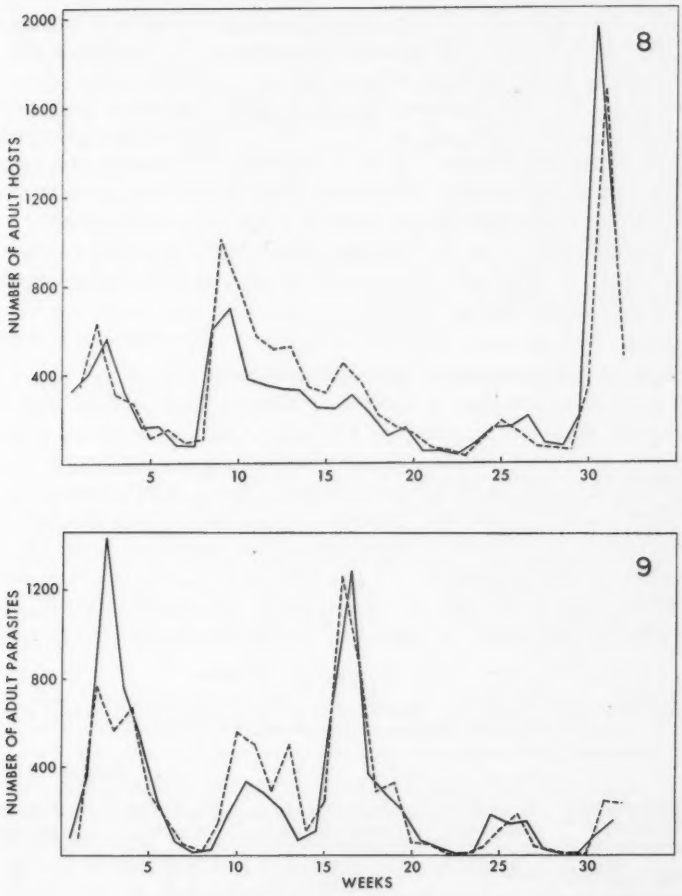


FIG. 6. Numbers of adult hosts and parasites counted on north and south benches of the experimental unit used for the propagation of population F. Solid line, hosts. Dotted line, parasites. N, north bench. S, south bench.

FIG. 7. Numbers of adult hosts and parasites counted on west and center benches of the experimental unit used for the propagation of population F. Solid line, hosts. Dotted line, parasites. W, west bench. C, center bench.

parasite population was quite variable during the first 4 weeks of the census period and this, no doubt, was caused by the irregular distribution of the original parasite females. The unusually high first peak of parasite numbers in one area (Fig. 7, W) explains why the second peak of parasite numbers for the experiment as a whole was not distinctly greater than the first (Fig. 4) in this one experiment out of the eight. A comparison during weeks 10–15 between the numbers of *T. vaporariorum* and *E. formosa* in Fig. 6 and also in Fig. 7 show that a higher density of hosts was accompanied by a higher



FIGS. 8 and 9. Numbers of adult hosts and parasites counted on the inside and outside rows of host plants on the north, west, and south benches of the experimental unit used for the propagation of population F. FIG. 8. Solid line, hosts counted on outside row of plants; dotted line, hosts counted on inside row of plants. FIG. 9. Solid line, parasites counted on outside row of plants; dotted line, parasites counted on inside row of plants.

density of parasites. As the immature hosts remain approximately where the adult deposited its eggs this suggests that the parasite population modifies its distribution with respect to that of its host.

Figure 8 compares the total numbers of adult *T. vaporariorum* counted on the inside and outside rows of host plants in three out of the four areas of population F. The numbers of the host on the two groups of plants were about equal except from the ninth to 16th weeks when there were more on the inside row of plants. Figure 9 compares the total numbers of adult *E. formosa* on the inside and outside rows of plants. During the fourth week there were many more parasites on the outside row than on the inside row; later, this relationship was reversed. A comparison during weeks 9–15 of Figs. 8 and 9 shows that higher densities of hosts on the inside row of tomato plants were accompanied by higher densities of parasites.

Figures 6, 7, 8, and 9 indicate that, in general, there was little variation in numbers of adult hosts and parasites which was related to the different areas of the experimental units. Most of the variation between areas occurred during the initial fluctuations; the rapid increase in host and parasite numbers at this time probably magnified any lack of uniformity in the original infestations. Variations in host distributions which arose at later periods were accompanied by a subsequent adjustment, to some extent, in the distribution of the parasite populations.

Host Plants

Throughout the experiments observations showed that at any given period a small number of host plants were more heavily infested with adults of *T. vaporariorum* than the majority of tomatoes. At the same time these or other plants were more heavily infested with parasite females than the remaining host plants. The data are not adequate for a comprehensive analysis of the distribution of host and parasite populations among the host plants but some indication of a non-uniform distribution is given in Table II. Table II gives the number of censuses for populations E, F, G, and H in which at least one-quarter of the total number of host and parasite adults counted in each census was located on only 1 of the 16 host plants. It is seen from

TABLE II

Number of samples in which one-quarter or more of adults are found on only 1 of the 16 host plants used in each of 227 censuses

	Host		Parasite	
Number of adults on 16 plants	Number of samples	Percentage of total samples	Number of samples	Percentage of total samples
1– 50	3	50	25	60
51– 100	5	31	8	32
101– 200	13	33	10	37
201– 500	22	32	16	28
501–1000	11	26	6	19
1001–	13	24	5	11
Total	67	30	70	31

Table II that in 30% (range 24–50%) of the 227 censuses one-quarter of the sample populations of *T. vaporariorum* was situated on one plant and that in 31% (range 11–60%) of the censuses one-quarter of the adults of *E. formosa* were found on one plant. Also, as the densities of host and parasite populations decreased, the percentages of samples having one-quarter of their adults located on one plant tended to increase.

There are, of course, a number of reasons why the host and parasite adults might be more numerous on a few plants than on the remaining ones. Some of these are chance dispersal, a tendency for the host, and therefore the parasite, to have a contagious distribution, the effect of host–parasite interaction on distribution of the two species, and the varying age of the host plants. Another important reason is that the host adults are differentially attracted to the host plants, probably because some plants are more succulent than the others. In this case the distribution of the host and parasite adults throughout the experimental area would be influenced by the distribution of the unusually attractive host plants throughout the experimental units.

Host plants which attract unusually large numbers of *T. vaporariorum* cannot be identified in advance. As one-half of the host plants in each experimental unit was replaced every month there was a constant rearrangement of the plants with varying degrees of attractiveness. In this case the location of the small number of exceptional plants was altered continually from one area to another of the experimental unit. This source of extraneous variation, therefore, was not associated with any particular point in the environment of the host and parasite species.

Contamination

A comparison of the numbers of hosts added and present in populations E, F, and H (Table I) reveals that host and parasite were already developing on the tomato plants at the beginning of the experiments. No effort was made to eliminate this natural infestation because it lengthened the time of initial increase of host and parasite populations and thus reduced the effect of life history phenomena on subsequent fluctuations. However, as four populations were propagated simultaneously the question arises as to whether the dispersal of host and parasite into experimental units continued for the duration of the experimental period. In this case the fluctuations of host and parasite would be modified by immigration or emigration of adults of both species.

It will be remembered that populations E, F, and H were started in late August with host plants grown during the summer months and population G about one month later. It is not surprising, therefore, that plants in populations E, F, and H were infested before the start of the experiments whereas, as shown in Table I, the numbers of adult hosts and parasites present in population G were closely related to the numbers introduced. Therefore, very few hosts or parasites were carried into the experimental unit on the host plants or migrated from contiguous populations at this most favorable time for dispersal after the start of the experiments. A careful examination of plants

subsequently transferred into the experimental sections revealed no specimens of either species. Also, though provision was made for mixing of some populations, the marked quantitative differences between the growth-forms of populations A and B and of populations C and D indicate a limited dispersal of host and parasite adults when the density of host plants was high. For these reasons it is probable that very few insects were added to the experimental populations after sampling began.

A potentially serious source of contamination was the immigration of other phytophagous insects into the experimental units. Constant surveillance of the tomato plants was necessary to suppress unidentified thrips and the two-spotted mite, *Tetranychus bimaculatus* Harvey.

Sampling

Although at least one-sixth of the host plants used in each experiment were examined to determine the growth-forms of host and parasite populations, the host plants were chosen in a systematic manner. Under these circumstances the sampling procedure was not entirely adequate statistically.

It is preferable, if possible, to record the total number of insects in experimental populations. However, in the present case this would have enabled only one experiment to be completed at a time, and because this host–parasite interaction is extremely sensitive and its results were unknown, it was desirable to record as many growth-forms as possible. As events turned out this was a fortunate choice, as considerable variation in growth-form occurred among the eight experiments.

There is little doubt that the dispersions of host and parasite populations greatly influence the growth-forms of these populations and should, therefore, be recorded as fully as possible. With the present host–parasite populations the entire host plant, not a leaf, forms the natural sampling unit because the host population gradually moves up the tomato plant. To be certain that host and parasite coexisted throughout the experimental area, each host plant was examined as often as possible—every third week in seven experiments, every second week in the eighth. It is remarkable that, in spite of this systematic change of sampling units, the trends of the fluctuations in each experiment were so smooth. It should be noted also that, with the possible exception of the parasite growth-form of population F, the number of hosts and of parasites at one peak of a fluctuation was greater for at least three consecutive censuses than the number at the maximum of the previous peak. There is little doubt that the trends in the fluctuations of the numbers of host and parasite were accurately described.

Conclusions

In the experiments to date the initial growth-forms of adult populations of *T. vaporariorum* and *E. formosa* were ones of fluctuations of slightly, but distinctly, increasing amplitude with successive minimal values being either approximately equal or slightly rising. During the 8-month period of the

experiments there were three fluctuations in the numbers of the host and two in those of the parasite. In some instances the second fluctuation in host numbers is tentatively considered to have comprised two separate peaks. The reasons for this interpretation are that the amplitude of the second of these peaks was quite variable in relation to that of the first and that there was only one instead of two peaks in parasite numbers in this portion of the growth-form of *E. formosa*. This variation in the amplitude of the second peak of the second fluctuation probably resulted from differences in the life cycles of the host and parasite species combined with some differences in the age structures of the populations at the beginning of the experiments. The fluctuations occurred in populations whose average densities ranged from high to very low. Though the general trend of *T. vaporariorum* and *E. formosa* populations was to fluctuate with peaks of increasing amplitude there was considerable variation, quantitatively, among the growth-forms of the eight populations. This is emphasized when the fluctuations of populations E and F, which were planned as replicates, are compared. The variation could have arisen from small chance events which had great significance at a later period, by differences in the numbers and time of introduction of hosts and parasites (note the differences in the original larval populations of populations E and F), or by very slight differences in the physical environments of the experimental units. In spite of the variation in the amplitude of the fluctuations between experiments, it is remarkable that the increase and decrease of the numbers of adult hosts and parasites became progressively greater within each experiment and occurred at relatively the same time between experiments.

During the period in which the growth-forms were recorded the rate of increase of host and parasite species was quite high. However, the density of the host was not sufficiently high to reduce its own rate of reproduction. This is apparent from the similarity of growth-forms obtained throughout the range of densities in populations A, B, C, D, and E. These densities were much lower than the 11,000 adult hosts, 1000 adult parasites, and many immature stages of both species which four tomato plants were able to support (2, Fig. 4). The sampling procedure gave a reliable indication of the fluctuations of the numbers of host and parasite species. Extraneous variation attributable to change in the physical and biotic environments, and migration between experimental units had, at the most, only a slight influence on the host and parasite populations. The fluctuations obtained in these preliminary experiments are, therefore, of internal origin and result from the properties of the host and parasite species.

Adults of *T. vaporariorum* live about four times as long as adults of *E. formosa* and so the growth-forms shown in Figs. 2, 3, 4, and 5 do not represent the proportion of each species which emerged from the host pupae. The interaction of host and parasite is actually between adult parasites and host larvae. In this case, the properties of the parasite species will have

to be determined eventually by investigating the variation in parasitism of the immature stages of the host as the densities of adult parasites and host larvae vary

The studies to date on the interaction of *T. vaporariorum* and *E. formosa* suggest that, although variations in host and parasite density modified the growth rates of host and parasite populations, the resultant fluctuations were not determined solely by these two agencies. The fluctuations appear to have been damped, the successive minima in each experiment never falling below its predecessor. The average densities of host and parasite in populations A, B, C, D, and E appear to have been related to the initial densities. The details of some growth-forms, as in populations E, F, and G, appear to have been related to the age structure of the host and parasite populations at the beginning of the experiments. In some populations greatly increased mortality of host larvae was associated with high parasite densities. It is apparent that larval mortality can be significant in large experimental areas as well as in very small ones. Whether or not it also plays a significant role at low average densities, as in populations F and H, remains to be determined but it is probable that host larval mortality is the critical factor in damping the fluctuations of *T. vaporariorum* and *E. formosa* populations.

Although the relations of host and parasite are very complex, the *T. vaporariorum* – *E. formosa* population is a suitable one for an empirical analysis of insect parasitism. It is necessary, first of all, to determine the influence of initial age structure and density of host and parasite populations on the subsequent fluctuations of both species. The variation in the parasitism of the immature stages of the host can then be investigated with particular reference to larval mortality. It is of interest to know not only the extent of larval mortality but also if it results from superparasitism or from the inability of second and third instars to withstand parasitization. It will also be necessary, of course, to propagate *T. vaporariorum* – *E. formosa* populations for periods much longer than 8 months under as uniform conditions as possible.

Acknowledgments

The author wishes to express his appreciation to Mr. G. D. Williamson and to Mrs. H. Clarke for assistance in this investigation.

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FURTHER OBSERVATIONS ON WATER ABSORPTION BY THE EGGS OF ACHETA DOMESTICUS (L.)[1]

J. E. McFarlane and C. P. Kennard[2]

Abstract

Eggs of *Acheta domesticus* (L.) absorb water during the stage of embryonic development in which the serosa surrounds the embryo and yolk. Water is absorbed over the entire surface of the shell. The egg membranes are more permeable to water, as shown by water loss in an unsaturated atmosphere, during the period of water absorption than either before or after; they are also permeable to dyes during the period of water absorption. The mechanism of water absorption is discussed. A possible explanation is presented for water loss after maximal absorption has occurred.

Introduction

Water absorption by the eggs of several species of crickets (*Acheta configuratus* (Walk.), *Gryllodes sigillatus* (Walk.), and both a Canadian and a Pakistani strain of *A. domesticus* (L.)) was described in a previous article (9). Further observations have been made on the water relations of the eggs of the Canadian strain of *A. domesticus*, and, in the present report, these observations are discussed in relation to the mechanisms of water absorption and loss.

Material

Methods for maintaining stocks of the Canadian strain of *A. domesticus* (5) and of obtaining and incubating eggs (9) have been previously described.

Embryology

Batches of 50 eggs were incubated on moist filter paper at 28° and 33° C. At daily intervals one batch from each temperature was selected at random and fixed in Bouin's solution. For the study of the embryo prior to the formation of the eye spots, eggs were stained *in toto* with borax carmine and destained in acid alcohol until only the embryo retained the stain.

Observations

The embryonic development of *A. domesticus* has previously been described by Heymons (6), and the following account of the timing of events during development at 33° and 28° C is based on his study.

The newly laid egg has an elongated rod-like appearance, and is usually convex ventrally and concave dorsally. The anterior pole is rather pointed while the posterior pole is rounded. During development the egg swells due to the absorption of water and, in most instances, retains its shape. However, the external shape is not a reliable guide to the orientation of the embryo

[1]Manuscript received September 15, 1959.
Contribution from the Department of Entomology, Macdonald College, McGill University, Macdonald College P.O., Que.
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within, as pointed out by Heymons (6), and at least the postrevolution stages represented in Fig. 1 do not have the most common orientation, in which the embryo is rotated to the left through 90° from its position in Fig. 1.

In the newly laid egg there is no sign of the germ band. It first becomes apparent at the posterior part of the convex ventral side after 24 hours of incubation at 33° C and 28° C (Fig. 1, A). At this stage it is relatively small, and extends scarcely one-third of the length of the whole egg. In the course of further development, the posterior end of the embryo pushes towards the dorsal side and after 48 hours at 28° C it has become hook-shaped (Fig. 1, B). The embryo subsequently becomes completely sunken within the yolk with the head facing posteriorly; this stage of development is reached after 48 hours at 33° C and after 72 hours at 28° C (Fig. 1, C). Heymons (6) has further shown that the amnionic folds have fused at this stage so that the embryo is covered on the ventral side by the amnion and the entire yolk is surrounded by the serosa. It is at about this stage that water absorption begins (Fig. 2).

Segmentation becomes apparent at the time of dorsal flexure and is completed by the time the embryo becomes stretched out on the dorsal side. The rudiments of the gnathal and thoracic appendages are also apparent at this stage. There are no further external changes in the embryo during this period; it merely increases in size (Fig. 1, D). The entire prerevolution phase takes 72 hours at 33° C and 120 hours at 28° C.

During the period of revolution, the embryo returns to the ventral surface of the egg, assuming its original position with the head facing anteriorly (Fig. 1, E). The revolution of the embryo is completed after 96 hours at 33° C, and 144 hours at 28° C.

The rotation of the embryo results in the rupture of the serosa. Heymons (6) showed that the serosa persists at the anterior end for a short time, but

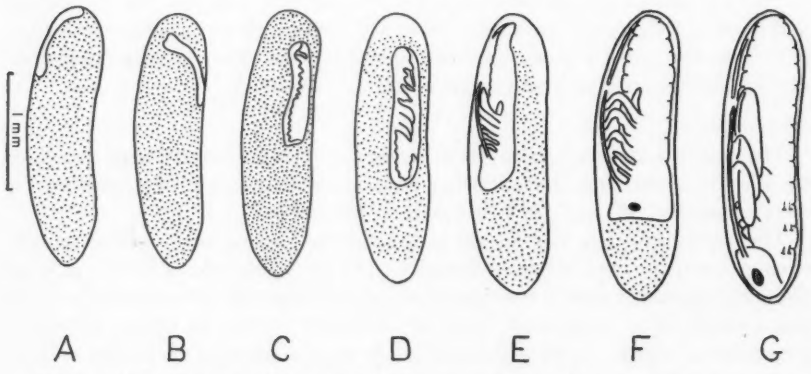

FIG. 1. (A) Lateral view of the egg of *A. domesticus* showing the germ band at the posterior end of the convex ventral side. (B) Commencement of dorsal flexure. (C) Embryo completely sunken within the yolk and segmentation completed. (D) Stage of embryonic development reached prior to revolution. (E) Revolution completed. (F) Dorsal closure and formation of eye spots. (G) Engulfment of yolk.

later sinks into the yolk and disappears. The amnion forms the provisional dorsal closure, but degenerates after final dorsal closure has taken place.

The dorsal body wall is completed after 120 hours at 33° C and 168 hours at 28° C. At this time also, the eye spots appear (Fig. 1, F). The embryo subsequently grows anteriorly absorbing the yolk, and by 144 hours at 33° C and 216 hours at 28° C, it occupies the whole egg (Fig. 1, G).

In Fig. 2, the time at which certain of these stages of embryonic development are reached is indicated on the curves for water absorption at 33° C and 28° C taken from McFarlane, Ghouri, and Kennard (9). The stage of embryonic development during which water is absorbed is that in which the serosa surrounds the embryo and yolk. This is probably generally true for insects (11). The serosa in *A. domesticus* also lays down a cuticle beneath the chorion.

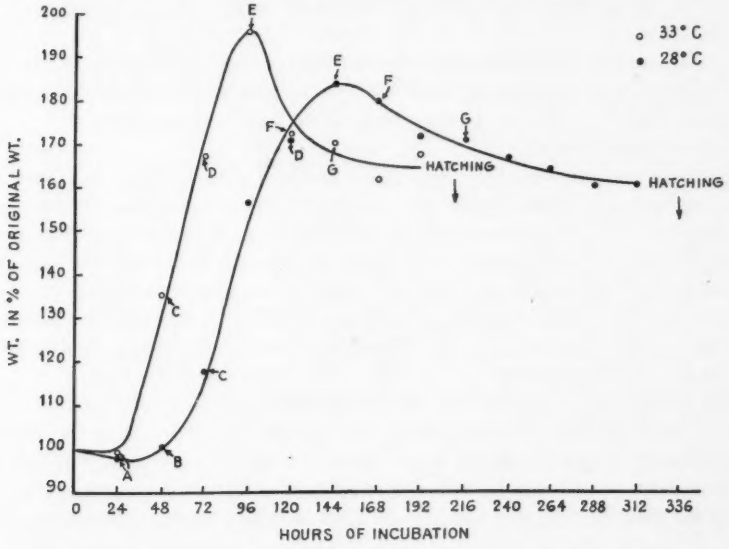

FIG. 2. Change in weight of eggs of *A. domesticus* incubated at 28° and 33° C on moist filter paper (from McFarlane, Ghouri, and Kennard (9)). The letters indicate the stages of development described in Fig. 1.

Site of Water Absorption

Water is taken up at the posterior end of the egg of locusts and grasshoppers through a specialized area called the 'hydropyle' (7, 9). We have found that coating either or both the anterior and posterior thirds of the eggs with liquid solder (which prevents water absorption in *Melanoplus differentialis* when it covers the hydropyle (10)) does not prevent water absorption, and in view of the fact that Heymons (6) has found no differentiated area in the serosa, apparently no specialized area corresponding to the hydropyle exists in *A. domesticus*, and absorption almost certainly occurs over the entire surface. Browning (2) similarly has found no evidence for a hydropyle in *A. commodus*.

Water Absorption from Glucose and Sodium Chloride Solutions

All experiments were carried out in the following way: The eggs were weighed on a torsion balance (capacity 50 mg) in lots of about 50 eggs each to determine the newly laid or 'original' weight; then incubated on moist filter paper at 33° C for 36 hours, i.e., until the eggs had just begun to absorb water; reweighed; transferred to the test solutions and incubated at 33° C for another 24 or 48 hours; weighed again; and then returned to moist filter paper for the remainder of the incubation period.

The eggs were immersed in either of two ways:

(*a*) In 1 ml of the test solution placed in the bottom of a 2-oz air-tight jar (diameter 4.6 cm). As the jars had a slightly concave bottom, the depth of solution varied, but maximum depth of the solution was about 0.3 mm.

(*b*) In 0.2 ml of the test solution placed in a small vial (diameter 1 cm), the vial in turn being placed in a 2-oz jar. Depth of solution was uniformly about 0.3 mm.

The percentage hatch showed considerable variation from lot to lot and from treatment to treatment, but the eggs incubated on moist filter paper showed a uniformly higher hatch (about 70%) than those immersed in the solutions (about 55%).

Less water was absorbed by eggs immersed in distilled water than by those remaining on moist filter paper for the period of treatment, and less water was absorbed by eggs immersed in water in the vials than by those in the jars (Tables I–VI). These results are no doubt due to the fact that the eggs immersed in distilled water are relatively deficient in oxygen as compared with those on the filter paper; the eggs immersed in water in the vials are similarly deficient as compared with those in the jars (the interfacial area in the latter case being 21 times greater).

Eggs immersed in 1 ml of the test solutions in the jars absorbed as much water from 1.2% NaCl, 1.6% NaCl, and 0.375 *M* glucose as from distilled water (Tables I and II). Little or no water was taken up from 0.5 *M* glucose in one experiment (Table I), and only slightly more from the same concentration of solution in a second experiment (Table II), in which little or none was

TABLE I

Water absorption from glucose and sodium chloride solutions. Eggs immersed in 1 ml of solution in jar

Treatment	Osmotic pressure (atm)	Wt. in % original wt.	
		After 36 hr	After 84 hr
1. Moist filter paper	—	111	190
Moist filter paper	—	114	194
2. Distilled water	—	110	162
Distilled water	—	112	173
3. 1.2% NaCl	8.4	120	169
4. 0.375 *M* glucose	8.4	112	171
5. 1.6% NaCl	11.2	111	132
1.6% NaCl	11.2	110	172
6. 0.5 *M* glucose	11.2	123	125
0.5 *M* glucose	11.2	122	126

TABLE II

Water absorption from glucose and sodium chloride solutions. Eggs immersed in 1 ml of solution in jar

Treatment	Osmotic pressure (atm)	Wt. in % original wt.	
		After 36 hr	After 84 hr
1. Moist filter paper	—	114	195
2. Distilled water	—	111	137
Distilled water	—	115	157
3. 1.6% NaCl	11.2	118	138
1.6% NaCl	11.2	109	167
4. 0.5 *M* glucose	11.2	110	133
0.5 *M* glucose	11.2	113	120
5. 0.625 *M* glucose	14.0	111	115
0.625 *M* glucose	14.0	112	121

TABLE III

Water loss by eggs immersed in 1 ml of 0.75 *M* glucose in jar

Treatment	Osmotic pressure (atm)	Wt. in % original wt.	
		After 36 hr	After 84 hr
1. Moist filter paper	—	112	198
Moist filter paper	—	116	196
2. Distilled water	—	110	130
Distilled water	—	110	176
3. 0.75 *M* glucose	16.8	108	99
0.75 *M* glucose	16.8	114	99

TABLE IV

Water absorption from sodium chloride solutions in jar and vial

Treatment	Osmotic pressure (atm)	Wt. in % original wt.	
		After 36 hr	After 84 hr
1. Moist filter paper	—	116	175
2. 1.2% NaCl—jar	8.4	112	134
1.2% NaCl—jar	8.4	109	167
3. 1.2% NaCl—vial	8.4	104	104
1.2% NaCl—vial	8.4	103	102

TABLE V

Water absorption from glucose and sodium chloride solutions in vials

Treatment	Osmotic pressure (atm)	Wt. in % original wt.	
		After 36 hr	After 60 hr
1. Moist filter paper	—	111	181
2. Distilled water	—	109	124
3. 1.2% NaCl	8.4	114	103
1.2% NaCl	8.4	108	100
4. 0.375 *M* glucose	8.4	106	106
0.375 *M* glucose	8.4	111	114

TABLE VI

Water absorption from glucose and sodium chloride solutions in vials

Treatment	Osmotic pressure (atm)	Wt. in % original wt.	
		After 36 hr	After 84 hr
1. Moist filter paper	—	100	178
2. Distilled water	—	112	129
3. 1.2% NaCl	8.4	106	100
1.2% NaCl	8.4	114	111
4. 0.375 *M* glucose	8.4	126	118
0.375 *M* glucose	8.4	112	109

absorbed from 0.625 *M* glucose. Water was lost when eggs were immersed in 0.75 *M* glucose. The eggs, therefore, appear to be in equilibrium with solutions having osmotic pressures of 11.2–14 atm. It is also apparent that osmotically equivalent solutions of NaCl and glucose are not isotonic. Finally, the eggs absorb as much water in 48 hours from solutions having osmotic pressures of 8.4–11.2 atm as they do from distilled water, although it should be noted that these are not rate measurements.

Whether or not water is taken up from NaCl and glucose solutions depends on the oxygen supply of the eggs. When the eggs are incubated in the vials they do not absorb water from 1.2% NaCl or 0.375 *M* glucose (Tables IV, V, and VI), and in fact lose water.

Permeability to Dyes

Eggs immersed in saturated solutions of basic fuchsin and cresyl violet during the period of water absorption gained weight, and developed and hatched normally. Examination of the yolk after the eggs had been returned to moist filter paper showed that the dyes had penetrated the egg membranes to stain the yolk.

Eggs which had been immersed in basic fuchsin were dissected just before hatching, and the gut contents of the mature embryos were found to be stained red. As the embryos were not stained, the cells of the embryo presumably metabolized the dye; the dye that remained in the yolk passed into the gut at engulfment.

'Active' Absorption of Water by the Eggs of Insects

It has been claimed for the eggs of *Locustana pardalina* by Matthée (8) that 'active' transfer of water into the egg is necessary to stretch the egg membranes, even though the osmotic pressure of the yolk is sufficiently high (11.92 atm) to accomplish this (3.88 atm required) if endosmosis alone is involved. Matthée rests his claim on the following experimental evidence:

(1) The osmotic pressure of the yolk of a 'turgid' egg is equivalent to that of a 1.7% NaCl solution, whereas 'turgid' eggs are in equilibrium with a glucose solution isosmotic with 2.1% NaCl; water absorption can take place against a concentration gradient and therefore must be, at least under this condition, 'active', and

(2) 'turgid' eggs placed in an atmosphere of nitrogen for 24 hours do not absorb water, whereas those in air do; that this is not due to a change in permeability of the eggs is shown by the fact that eggs placed in a dry atmosphere of nitrogen lose water at the same rate as eggs in a dry atmosphere of air (over a 24-hour period).

Matthée's experiments in part have been responsible for the belief that 'active' transfer is the usual mechanism of water absorption by insect eggs (3), even though osmotic gradients exist (7, 8) which could account for the water movement. It is clear, however, as will be shown, that his experiments were not decisive, and that the case for 'active' transfer has yet to be proved.

Matthée's comparison of the osmotic pressure of a 'turgid' egg with the osmotic pressure of the glucose solution in equilibrium with a 'turgid' egg 21 hours after immersion is not valid, as it neglects the fact that the egg was developing during the period of the experiment. Laughlin (7) has shown for *Phyllopertha horticola* that the osmotic pressure of the yolk and extra embryonic fluid drops during the rapid absorption period from about 13 atm to about 8 atm in the swollen egg, at the same time as the egg is absorbing 5 times the quantity of water present in the egg before swelling, and he concludes that "it is obvious that the embryo is controlling the concentration of soluble material in the yolk and extra embryonic fluid, perhaps by the breakdown of large molecules or by the 'binding' or utilization of incoming water". It is, therefore, the osmotic pressure of the yolk of eggs which have been found experimentally to be in equilibrium with a particular concentration of solution that should be used for a relevant comparison.

The possibility that the egg is permeable to the ions or molecules used in this kind of experiment cannot be neglected, as penetration of these particles would increase the osmotic pressure within the egg. As we have shown, eggs of *A. domesticus* are permeable to dyes.

The 'proof', in Matthée's view, that the permeability of the egg to water was unaffected by the nitrogen atmosphere is the fact that the rate of water loss from 'turgid' eggs was unaffected by an atmosphere of nitrogen, as measured over a 24-hour period. It is pertinent to ask to what extent this loss may be accounted for by loss from the shell. If the embryo, yolk, and hydropyle cells contributed little to this loss, then a decrease in permeability of the hydropyle cells (and the point at issue here is whether or not such a decrease occurs) would not appreciably affect the water loss in a dry atmosphere.

Matthée studied the change in rate of water loss with time of 'turgid' eggs in dry air and found that the rate decreased rapidly during the first few days and then less rapidly, reaching a minimum rate at the eighth day and remaining more or less constant at this minimum level for another 7 days. He explains the decrease in the evaporation rate as being "presumably due to the lowering in moisture content of the eggs and the drying out of the hydropyle membranes." Now water loss due to the drying out of the hydropyle membranes must have been maximal during the first day, and it was over this same period that water loss in nitrogen was determined. As it is not known to what extent this drying out contributes to the water loss, one cannot conclude from

Matthée's experiment either that the permeability of the hydropyle cells remains the same or decreases during exposure to an atmosphere of nitrogen.

In addition to Matthée's experiments, high Q_{10} values (10–12) for water absorption have been cited (3) in support of the 'active' transfer hypothesis, the high Q_{10} values implicating a chemical rather than a physical process. However, Laughlin (7) has shown that the developing embryo controls the osmotic pressure within the egg. The rate of water absorption will, therefore, depend on the rate at which the embryo develops, but it is not necessary to postulate anything more than osmosis to account for the actual entry of water.

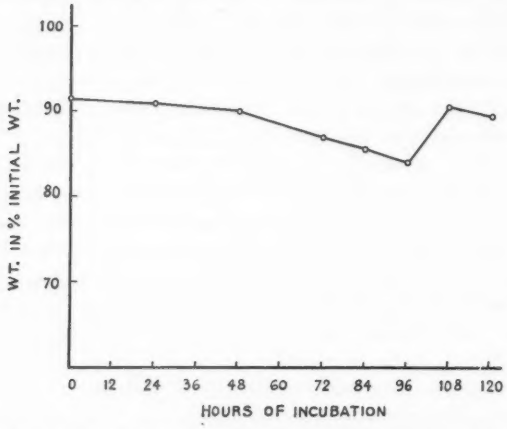

FIG. 3. Water loss by the eggs of *A. domesticus* at various stages of development during 24 hours' exposure to 95% R.H. Temperature 33° C.

Water Loss in Unsaturated Air

Water loss by the eggs at 95% R.H. and 33° C over a 24-hour period was determined at daily intervals during development at 33° C on batches of 50 eggs. The results (Fig. 3) show that the permeability of the egg increases progressively during the period of water absorption, diminishing rather abruptly thereafter but not returning to the low level of the newly laid egg. These results are admittedly not an accurate measure of the permeability changes, but they do indicate clearly enough that such changes do occur in the developing egg, and are in the same direction as those found by other workers (2, 7).

Water Loss after Water Absorption

A loss of water from the eggs of crickets occurs just after maximal absorption of water (9, Fig. 2). As this loss occurs against a concentration gradient, it must be considered an 'active' process, and it can perhaps be explained as follows:

The serosa in *A. domesticus*, as in most insects, lays down a cuticle beneath the chorion. This cuticle (as well as the chorion) must be permeable to water

during the period of water absorption, as water absorption begins with the formation of the serosa and ends with its rupture during revolution.

On the completion of water absorption in *A. domesticus*, the egg becomes less permeable to water, as shown by water loss in unsaturated air, and actually loses water; but there is no further gain in water.

We have found that eggs of *A. domesticus* will blacken, presumably due to melanin formation, within 12 hours if immersed in tyrosine 24 hours after laying or at any time thereafter. A tyrosinase is therefore present in the chorion during the whole of embryonic development.

Now if, at the completion of revolution, the shell were to tan:

(1) It would become saturated with water, and entry of water into the egg would at least temporarily cease (1) for this reason;

(2) The shell of the egg would become more rigid, perhaps sufficiently to prevent any further absorption of water. And, if water were lost by the egg in an unsaturated atmosphere, a suction pressure would be created, due to the rigid shell, which would tend to oppose further water loss: this could possibly account for the decreased permeability of the egg after water absorption has taken place;

(3) Water could actually be lost by the egg, either during the tanning process itself, i.e., from the cuticle, as water loss is known to accompany tanning (4), or supervening on tanning due to the increased hydrostatic pressure within the egg.

Water loss after water absorption has been found to occur only in crickets (9), but may occur in *Phyllopertha* (and other insects) which, like crickets, absorbs water over the entire surface of the egg. However, as the egg of *Phyllopertha* is much larger than that of crickets, it has a lower surface/volume ratio, and this could have prevented the detection of water loss by Laughlin. One would not, of course, expect to find this loss in eggs which absorb water through a hydropyle.

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UNIDENTIFIED GROWTH FACTORS IN BREWER'S YEAST

I. NECESSITY OF THESE FACTORS FOR TRIBOLIUM CONFUSUM DUVAL[1]

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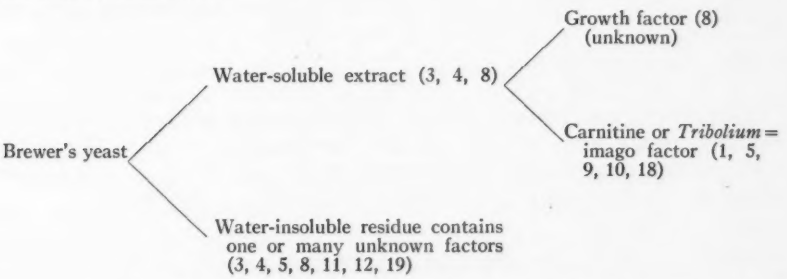
Abstract

The presence in brewer's yeast of unknown growth factors necessary for the normal development of *Tribolium confusum* Duval is confirmed. Casein appears to contain some of these unknown factors. A synthetic diet suitable for the study of these factors is presented.

Introduction

It has been known for a long time that brewer's yeast contains some unknown factors indispensable for the normal development of *T. confusum*. Our present knowledge on these new and unknown factors is shown below. Interesting reviews on the subject were presented by Magis (17), French (8), Lipke and Fraenkel (16).



The present series of experiments was initiated with a view to obtaining data on these different factors. At first, an attempt was made to determine the relation between yeast, vitamin mixtures, and the quantity of casein in a synthetic diet. Knowing these relations, we have composed a basic diet to be used in our future work. Such relations have already been studied by other authors using *T. confusum* (8). It was believed important to repeat these experiments with our strain of *T. confusum* since Fraenkel and Leclerq (6) have shown that different strains of a given insect species can react differently to the same diet.

Experimental Methods

Details of the technique for using *T. confusum* in nutritional experiments have already been published (13, 14). The composition of the basic diets and vitamin mixtures is described in Tables I and II.

[1]Manuscript received October 27, 1959.
Contribution from the Department of Biochemistry, School of Medicine, Laval University, Quebec. This work was supported by a grant from the "Conseil des Recherches Agricoles, Ministère de l'Agriculture, Québec".
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TABLE I

Composition in the basic diets in %

Ingredients	R 1	R 2
Casein*	48.0	20.0
Glucose	47.0	75.0
Cholesterol	1.0	1.0
Salt mixture No. 2 U.S.P. XIII	4.0	4.0

*Vitamin-free casein—Nutritional Biochemicals Corporation, Cleveland, Ohio.

TABLE II

Composition of the vitamin mixtures, μg/g of diet

Vitamins	M.V. 1	M.V. 2
Thiamin HCl	25.0	1.0
Riboflavin	12.5	2.0
Pyridoxine HCl	12.5	1.0
Nicotinic acid	50.0	8.0
Calcium pantothenate	25.0	4.0
Biotin	0.5	0.05
Folic acid	2.5	0.2
Choline chloride	500.0	1500.0
Inositol	250.0	—

Fifty newly emerged larvae (0 to 15 hours of age) obtained from our permanent culture were divided into five batches of 10 larvae. These larvae were put into small vials containing 3 g of diet and the vials were kept in a room maintained at $28 \pm 1°$ C with relative humidity at $70 \pm 5\%$.

Results and Discussion

The results shown in Table III indicate the importance of the yeast in the nutrition of *T. confusum* larvae. With any of the diet used, the addition of 5% yeast always gives results comparable to those obtained with whole wheat

TABLE III

Importance of yeast in the larval growth of *T. confusum* on different basic diet

Basic diet No.	Vitamin mixture No.	Brewer's yeast, %	Number of pupa out of 50 larvae	Larval period in days	
				Mean ± σ_M	Spread
1	—	5	42	21.6±0.11	21–24
2	—	5	44	21.3±0.15	20–23
1	1	—	47	19.9±0.16	19–22
2	1	—	44	32.9±0.45	28–37
1	2	—	42	27.6±0.20	24–31
2	2	—	39	34.4±0.14	29–42
1	1	5	49	16.3±0.08	16–19
2	1	5	49	17.5±0.10	17–19
1	2	5	47	18.4±0.12	18–21
2	2	5	48	19.4±0.26	18–22
Whole wheat flour*			49	17.2±0.13	17–20

*Rearing diet.

flour. This is in complete agreement with the data already published by different authors (5, 8, 14, 19) working on other strains of *T. confusum.*

In the basic diet number 1 (48% casein) and the vitamin mixture number 1, the larvae reach the pupal stage in 19.9 days. In the basic diet number 2 (20% casein) and the same mixture of vitamins number 1, the larvae need 13 days more to complete their growth, i.e. 33 days. It would seem then that 20% casein is not sufficient to meet the requirements of *T. confusum* larvae.

The vitamin mixture number 2 is an optimum–minimum mixture composed from the data of Fraenkel and Blewett (5), and Lemonde and Bernard (15). The vitamin mixture number 1 is the optimum mixture described by French (8). It contains an excess of vitamins. If we utilize the minimum vitamin mixture number 2 with the basic diet including 48% casein, the larval growth is maintained for 27.6 days, that is 7 days more than with the vitamin mixture number 1. We think this difference of 7 days is due to the low quantity of nicotinic acid of the vitamin mixture number 2. Fraenkel and Stern (7) have found that the nicotinic acid requirements of *T. confusum* increase with increasing protein content of the diet in a fashion whereby they are roughly doubled with a doubling of the amount of protein.

With the basic diet containing 20% casein and the minimum vitamin mixture number 2, the larvae reach the pupal stage in 34 days. Thus, there is no difference between the results obtained with the two mixtures of vitamins if the diet encloses 20% casein.

From these results, we can conclude that the larvae grow more rapidly on a diet containing 48% casein. This difference in growth is apparently not due to the greater quantity of casein in this diet. As a matter of fact, with 20% casein and 5% yeast the larvae grow as rapidly as on a diet containing 48% casein. Since the quantity of proteins supplied by 5% yeast is certainly not equivalent to that present in 28% casein, it seems, therefore, that certain of these unknown growth factors of the yeast are also present in our casein.

Chirigos (2) showed that the optimum level of casein for the larvae of *T. confusum* is between 24% and 30%. According to this author, the larval stage was longer in a diet including 50% casein, because of the toxic effects of certain amino acids, minerals, or other factors present in casein. The same author says that casein includes none of these unknown factors of the yeast. This divergence of opinions is due to various reasons. Firstly, the casein used is not the same as used by all the authors. Chirigos (2) utilizes a Labco free-vitamin casein tested by the Rutgers Bureau of Biological Research. French (8) uses a vitamin-free casein, biologically assayed, coming from Labco Brand, Borden Company, New York. Our casein is also a vitamin-free casein, but was supplied by the Nutritional Biochemicals Corporation, Cleveland, Ohio. A second possible reason should be a difference between the strains of *T. confusum.* Fraenkel and Leclerq (6), working with *Tenebrio molitor,* have demonstrated that the quality of casein in the diet appears to be of prime importance. They have also proved that different strains of this insect react differently to a deficiency of carnitine in the diet.

Because of these results, we have chosen the basic diet number 2 and the vitamin mixture number 2 for the study of these unknown factors present in brewer's yeast. This diet permits the easy detection of the nutritive effect of the presence or absence of the yeast's unknown factors.

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DESCRIPTIONS OF TWO NEMATODES, EKTAPHELENCHUS MACROSTYLUS N. SP., AND LAIMAPHELENCHUS ULMI N. SP., WITH A KEY TO SPECIES OF LAIMAPHELENCHUS[1]

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Abstract

Two new species of nematodes from Canada are described. Females of *Ektaphelenchus macrostylus* n. sp. were collected from the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk., and males from the beetle galleries in the bark of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco. *Laimaphelenchus ulmi* n. sp. was collected from bark of elm, *Ulmus americana* L. A key is given to the species of *Laimaphelenchus*.

Introduction

Ektaphelenchus macrostylus n. sp. was collected during a study of nematodes harbored by the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk., from Vernon, B.C. Only the females of this nematode were seen on the beetles, under the elytra; a search for the males was extended to the beetle galleries, frass, and bark of the Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, and they were finally located in the bark.

Laimaphelenchus ulmi n. sp. was collected from the bark of elm, *Ulmus americana* L., growing on the Central Experimental Farm, Ottawa.

Ektaphelenchus macrostylus new species

(Figs. 1–5)

Measurements

Female

Length 0.7 mm; $a=35$; $b=4$; $c=23$; $V=79\%$.

Male

Length 0.7 mm; $a=34$; $b=3.7$; $c=18$; $T=30\%$.

Description

Adult dull white in color with cylindrical body tapering at both ends and assuming a characteristic shape (Figs. 1–2) when killed in gently heated water. Cuticle with regular and distinct annulations and faint longitudinal markings. Lateral fields with three lines. Head offset by slight constriction, flattened, and slightly widened at top. Six lips fused together and lightly sclerotized. Styletiform stoma. Stylet a prominent feature of anterior part of the nematode; 14μ long, pointed, and with small basal knobs. Strong extensor muscles attached to cephalic walls just behind head. Anterior portion of esophageal lumen heavily lined for a short distance of 10–12μ and appearing like a posterior extension of the stylet. Esophageal precorpus long and slender with slight constriction just before joining elongated and ellipsoidal

[1]Manuscript received September 24, 1959.
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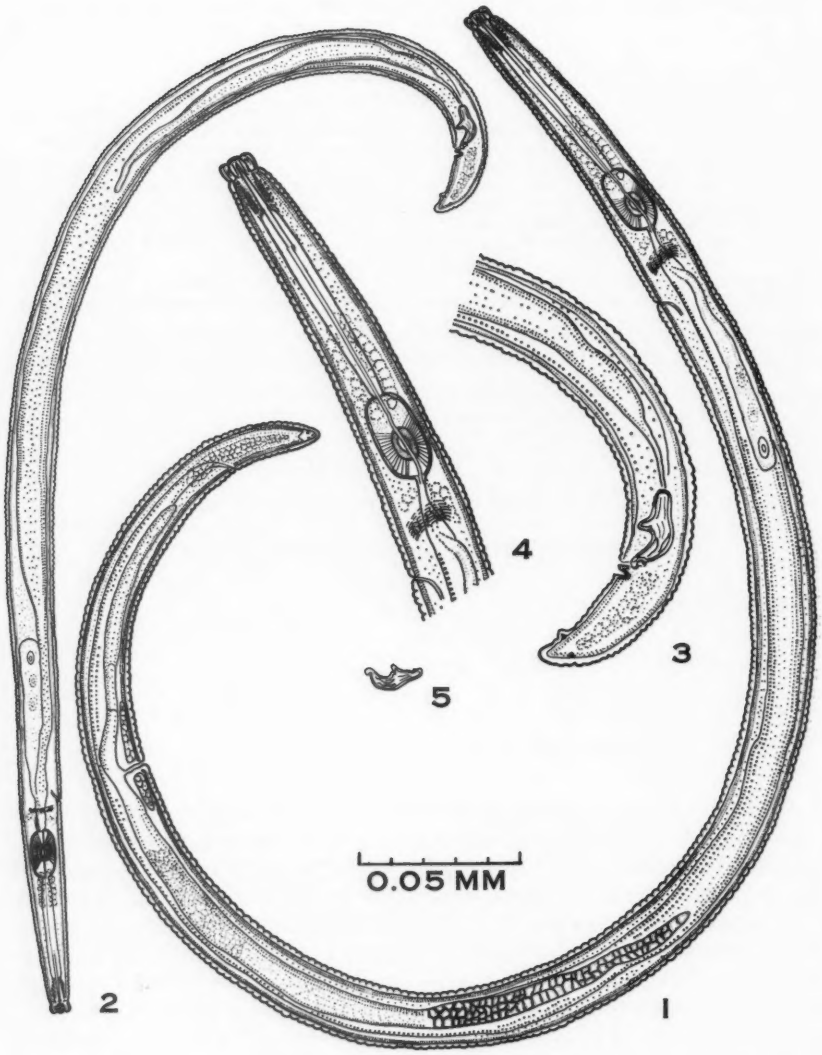


FIGS. 1–5. *Ektaphelenchus macrostylus* n. sp. 1. Female adult. 2. Male adult. 3. Male tail. 4. Anterior end of female. 5. Spicule.

median bulb, which is twice as long as wide and has a fibrillar appearance with semicircular valves. Esophageal bulb nearly two-thirds as wide as body in this region. Nerve ring crossing narrow isthmus about five to six annules behind bulb. Posterior esophageal gland nearly as long as median bulb and precorpus. Excretory pore just posterior to nerve ring. Intestine with wide lumen and granular in appearance.

Female with a transverse vulva with a raised border. Ovary single and outstretched, postuterine branch long and covering nearly two-thirds of distance between vulva and tip of tail. Rectum and anus faintly visible. Anal pore not discernible in every specimen. Tail tapering to a rounded, blunt end, which in a few specimens has a short, subterminal mucro. Tip of tail not annulated. Cuticle on sides of tip of tail with a rough and eroded surface and a few fine bristles.

Male with a single, outstretched testis that does not reach middle point of nematode body. Spicules very conspicuous, mitten-shaped with a prominent ventral process. Distal ends of spicules notched dorsally. Viewed laterally, cuticle with a well-defined notch just posterior to anus. Gubernaculum and caudal bursa missing. Tail narrowing gradually to a smooth, rounded tip without annulations; a pair of caudal papillae just anterior to tip.

Diagnosis

This species closely resembles *E. obtusus* Massey (4), from which it differs in having basal knobs on the stylet and three lines in the lateral fields. Further, males of *E. macrostylus* have a hooklike notch on the distal end of each spicule and have only one pair of caudal papillae.

Habitat

Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk., for females, and bark of *Pseudotsuga menziesii* (Mirb.) Franco for males.

Type Locality

Vernon, British Columbia, Canada.

Type Specimen

No. 393, Canadian National Collection of Nematodes, Ottawa, Ontario.

***Laimaphelenchus ulmi* n. sp.**

(Figs. 6–11)

Measurements

Female

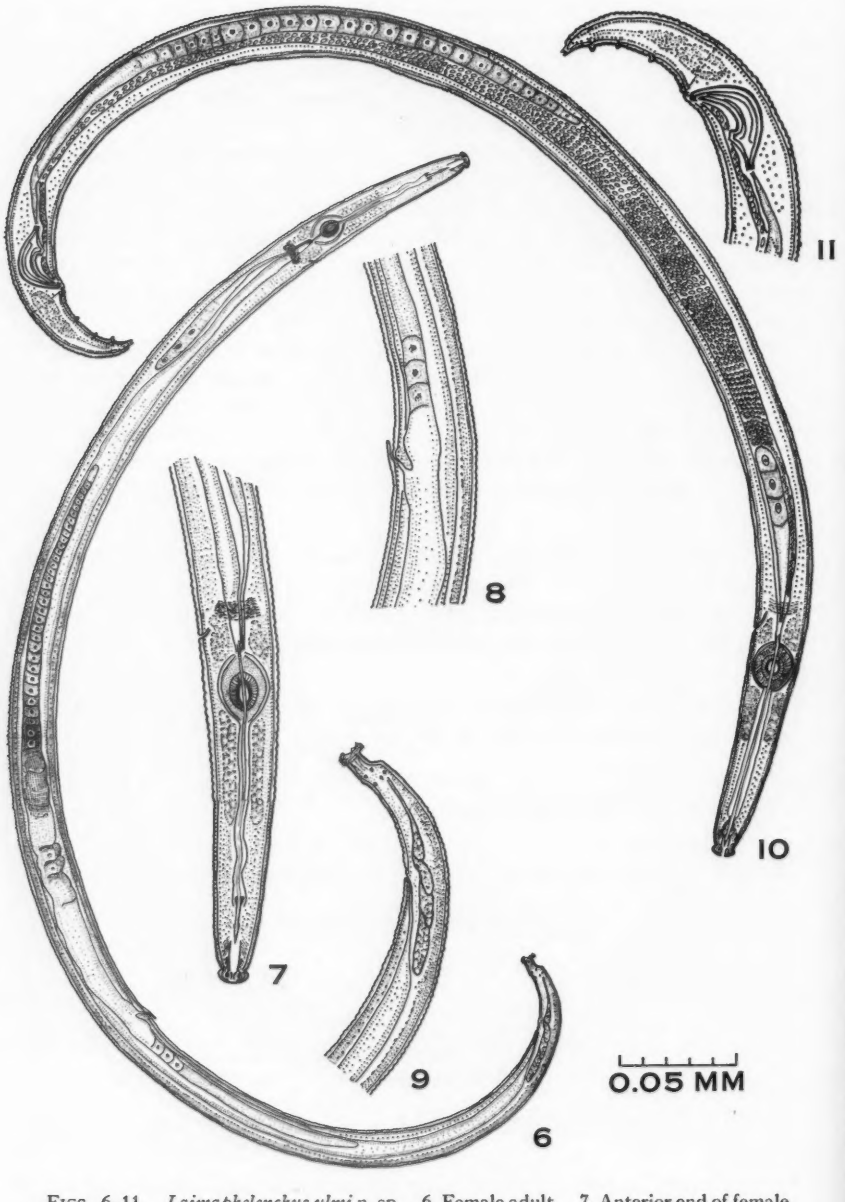
Length 0.71 mm; $a=32.5$; $b=4.35$; $c=20.3$; $V=65\%$.

Male

Length 0.44 mm; $a=27$; $b=4$; $c=12.3$.

Description

Cuticle finely annulated, with longitudinal markings. Head and tip of tail without annulations. Lateral fields showing two lines running from region of esophageal bulb to tip of tail. Head distinctly offset, arched proximally,



FIGS. 6–11. *Laimaphelenchus ulmi* n. sp. 6. Female adult. 7. Anterior end of female. 8. Vulva. 9. Female tail. 10. Male adult. 11. Male tail.

and moderately sclerotized. Stylet 11μ long, jointed, and with basal knobs tapering upwards. Precorpus of esophagus long and slender, with a fibrillated spheroidal bulb with a sclerotized valve. Nerve ring not very prominent in mounted specimens and crossing isthmus a short distance from median bulb. Excretory pore midway between esophageal bulb and precorpus.

In female, vulva covered by a cuticular flap directed posteriorly. Vagina slanted anteriorly. Ovary single, outstretched, falling short of posterior esophageal gland by about 2½ times body width. Postuterine branch reaching halfway from vulva to anus. Anal pore visible. Tail with rather remarkable features: tapering slightly throughout, then abruptly constricting and terminating in four small tubes; ends of tubes open, fringed by very minute bristles. A few bristles also visible in region preceding abrupt constriction in tail.

Male much smaller than female. In specimens examined, gut of male engorged with elliptical, unicellular, and refractile particles throughout its length. Testis single, outstretched, and limited to posterior half of body. Spicules mitten-shaped with distal ends bent inwards, and, in lateral view, have long and deeply arched shafts. Spicules nearly two-thirds as long as tail. Gubernaculum and bursa missing. Tail with three pairs of papillae and gradually tapering to tip, which shows abrupt constriction and has two fine bristles at the terminus.

Diagnosis

The anatomical features that distinguish *L. ulmi* n. sp. from *L. moro* Fuchs, 1937, are: (*a*) mitten-shaped spicules like those seen in Aphelenchinae, with ventral process on shaft, and neither wavy nor curved and without ventral process on shaft like those in Tylenchidae and drawn and described by Fuchs (2), (*b*) male tail abruptly constricted at tip, with two fine bristles as in female and not terminating in a bluntly rounded tip, (*c*) female tail terminating in four tubes instead of three, and (*d*) lateral fields with two lines. These nematodes were collected from the bark of elm trees and not from the bark beetle.

L. ulmi differs from *A. penardi* in having (*a*) head knob-shaped, set off with a slight constriction from the rest of the body, (*b*) stoma wide, tubular, (*c*) basal knobs of spear pear-shaped, tapering anteriad along shaft, not rounded, and not limited to base of spear, (*d*) in female, vulva covered by a cuticular flap, (*e*) in male, processes at tip of tail not tubular, and spicules mitten-shaped, with distal ends bent inwards, and, in lateral view, spicule shafts appearing long and deeply arched.

Habitat

Bark of *Ulmus americana* L.

Type Locality

Ottawa, Ontario, Canada.

Type Specimen

No. 1440, Canadian National Collection of Nematodes, Ottawa, Ontario.

Discussion

The genus *Laimaphelenchus* and the subgenus *Ektaphelenchus* were described by Fuchs (2). Later (1, 3, 5) *Ektaphelenchus* was raised to a genus and, according to Ruehm (5) includes 10 species, namely, *tuerkorum*, *betulae*, *scolyti*, *dendroctoni*, *goffarti*, *cunicularii*, *amitini*, *typographi*, *tenuidens*, and *alni*. In addition to these, Massey (3) described *E. obtusus*, which brought the number of species to 11.

The genus *Laimaphelenchus* and its type and only species, *L. moro*, were considered synonymous with *Aphelenchoides* and *A. penardi* by Goodey (3). It was obvious during the present study that the three nematodes *moro*, *penardi*, and *ulmi* have anatomical characters that distinguish them, but also certain common features that link them into an identifiable group under the genus *Laimaphelenchus*. The latter features are: a wide stoma, a stout stylet with basal knobs, characteristic appearance of the tip of the tail, and a cuticular flap on the vulva in *moro* and *ulmi*. *Laimaphelenchus* is recognized as a valid genus and includes the following three species:

Laimaphelenchus Fuchs, 1937.

L. moro Fuchs, 1937. Type species.

Syn. *Aphelenchoides moro* (Fuchs, 1937) Goodey, 1951.

L. penardi (Steiner, 1914) Skrjabin *et al.*, 1954.

Syn. *Aphelenchus penardi* Steiner, 1914.

Aphelenchoides penardi (Steiner, 1914) McBeth, 1937.

L. ulmi n. sp.

KEY TO SPECIES OF *LAIMAPHELENCHUS*

MALES

1. Spicules wavy, without ventral process 2
   Spicules mitten-shaped, with ventral process 3
2. Tail tapering slightly to a rounded tip ..... *moro* Fuchs
   Tail flat, with sharp constriction 3
3. Tip of tail with four tubular processes ..... *penardi* McBeth
   Tip of tail with two bristles ..... *ulmi* n. sp.

FEMALES

1. Head narrow, flat, and contiguous with neck; basal knobs of stylet terminal and rounded 2
   Head knob-shaped and set off; basal knobs of stylet tapering along shaft 3
2. Vulva without a cuticular flap ..... *penardi* McBeth
   Vulva with a cuticular flap 3
3. Tip of tail terminating in three tubes ..... *moro* Fuchs
   Tip of tail terminating in four tubes ..... *ulmi* n. sp.

Acknowledgment

I wish to acknowledge with thanks the help received from Dr. A. D. Baker, Chairman, Nematology Section, Entomology Research Institute, Ottawa, in planning these studies and in preparing the manuscript.

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POPULATION PROCESSES IN THE VOLE AND THEIR RELEVANCE TO GENERAL THEORY[1]

DENNIS CHITTY

Abstract

No animal population continues to increase indefinitely, and the problem is to find out what prevents this. Increase among voles is halted by declines that recur fairly regularly, and can be identified by certain associated characteristics as belonging to a single class of events. By examining enough of these instances, and contrasting them with control populations that are expanding, conventional types of answer to the problem can be eliminated. According to field evidence the individuals in a declining vole population are intrinsically less viable than their predecessors, and changes in the severity of their external mortality factors are insufficient to account for the increased probability of death. On the assumption that vole populations are a special instance of a general law, the hypothesis is set up that all species are capable of regulating their own population densities without destroying the renewable resources of their environment, or requiring enemies or bad weather to keep them from doing so. The existence of such a mechanism would not imply that it is always efficient, especially in situations to which a species is not adapted, or that species do not also occur in environments where the mechanism seldom, if ever, comes into effect. The hypothesis states that, under appropriate circumstances, indefinite increase in population density is prevented through a deterioration in the quality of the population. The hypothesis can be falsified, or shown to be irrelevant to a particular situation, by proving that there are no significant differences between expanding, stationary, and declining populations in the distribution of the properties of the individuals. Tests of this hypothesis are relevant to all theories about the factors limiting animal numbers, since the effects of most mortality factors depend upon properties of the organisms, and it cannot safely be assumed that so important an environmental variable as population density has no effect on the physiology of the individual or the genetics of the population. Contrary to the assumption often made, it is therefore a priori improbable that the action of the physical factors is independent of population density. It is therefore postulated that the *effects* of independent events, such as weather, become more severe as numbers rise and quality falls. This hypothesis, if true, overcomes two of the difficulties often met with in population studies: that there is no consistent evidence of (*a*) the mortality factors that are themselves influenced by population density in the manner required by one system of thought, or (*b*) the climatic catastrophes required by other systems.

Introduction

Populations of animals such as the vole (*Microtus agrestis*), which fluctuate more or less regularly in numbers, are not easy to fit into general theories about the natural regulation of animal numbers. This must mean either that these species are exceptional, and facts about them can safely be ignored, or else that the theories need to be modified, which is the alternative discussed in the present paper.

Defining the Problem

The object of many field studies is to find out (1) why population density does not go on rising indefinitely, and (2) why it varies from one type of environment to another. The first enquiry concerns a common property of all populations, the second concerns differences between them. To illustrate these definitions we may imagine two different types of environment, each

[1]Manuscript received October 5, 1959.
Contribution from Bureau of Animal Population, Department of Zoological Field Studies, Oxford University, Oxford, England.

starting off with the same low numbers of a certain species of animal (Fig. 1, A and B). We may also suppose that both populations expand at different rates and eventually maintain different levels of abundance. We need to explain why the populations are alike in failing to keep up their initial rates of increase, and why they differ in the levels attained.

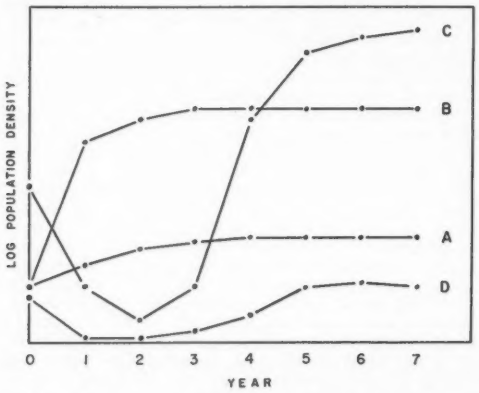

FIG. 1. Curves A–D show the results of an imaginary annual census of four populations occupying different types of habitat. The problem is to find out if there is a common explanation for failure of the populations to go on increasing indefinitely. Comparisons may be made within areas at different times (B in year 0–1 compared with year 4–5, for example) and between areas at the same time (A and B compared with C and D in years 0–1 or 4–5, for example). The present paper is not concerned with methods of enquiry into the differences between good and bad habitats (B and C compared with A and D).

The reason for recognizing two distinct problems is that it is not necessarily true, as is sometimes assumed, that factors associated with a difference in abundance between populations also prevent unlimited increase within them. Differences in food supply, weather, parasitism, etc. are often associated with differences in abundance, but we cannot infer that these are the factors preventing unlimited increase. Or to reverse the argument, although epidemics, or frosts, may not normally prevent unlimited increase, we cannot infer that such factors do not affect abundance. These matters must be decided empirically.

In the present paper I shall discuss only the first of the two problems mentioned above, which is seen as an attempt to discover the factors associated with failure to go on expanding at the higher rates observed within habitats, these rates being regarded as a control for all other rates of change. For proper controls, however, observations must also be made on a random selection of populations that are expanding at the same time as those that are not (Fig. 1).

Few populations are likely to conform to the simplified pattern so far imagined; indeed the majority will have more or less severe or prolonged declines, which may or may not have to be taken into account when searching for the factors that prevent unlimited increase. Population density may be

expected to fall if the resources of the environment are reduced or the climate deteriorates, and an instance of this sort would be a special case of the second of the two problems; but other declines, whether they occur at regular or irregular intervals, must be considered as possible instances, in more obvious form, of the general phenomenon of failure to maintain a high rate of increase. Thus the problem is here assumed to be the same whether an initial rise in numbers is followed by a systematically recurring decline, irregular fluctuations, or a more stationary state, and the sharp distinctions drawn by some authors are not accepted.

Just as no single factor is associated with all differences in average abundance, no single factor is likely to be common to all departures from the maximum observed rates of increase. We must nevertheless avoid giving different explanations for effects that merely differ superficially, but are in fact the same. It is fortunate that in voles a specific kind of decline can be recognized, not merely by its typical periodicity, but by various other criteria, especially those based on the distribution of body weight (4, 31). Given enough instances of a recognizable class of events it is then possible to replace unfalsifiable propositions about *some* declines by hypotheses about *all declines of a certain kind*, and hence to eliminate explanations that do not fit the facts in every case.

Throughout this paper it will be assumed that we are dealing with closed systems. As will become clear, the conclusions arrived at would not apply to populations recruited from areas in which there were fundamental differences in the conditions determining population density.

Evidence Against Proposed Hypotheses

The present section gives reasons for rejecting some of the explanations that have been advanced to explain recurrent declines in numbers in voles. The observations were made at Lake Vyrnwy in Wales, in 1936–9 (4, 5), and 1945–59 (largely unpublished).

(*a*) *Infectious Disease*

The most reasonable hypothesis consistent with the facts available before 1938 was that overcrowded vole populations were regularly decimated by epidemics. However, the lack of any consistent association between infectious disease and population changes (14), and the discovery of vole tuberculosis (30), helped to destroy the basis for the belief that the populations were controlled by epidemic diseases. In one part of Lake Vyrnwy two-thirds of the animals were tuberculous at a time of severe mortality. This association of circumstances was similar to others described in the literature; but since several independent populations were being studied at Lake Vyrnwy it could be shown that the association was not invariant (5). The disease was not a necessary condition for the decline of the populations, as it was almost entirely absent from other areas from which they disappeared; nor was it a sufficient condition, as some populations remained abundant in spite of being heavily infected.

Field work on tuberculosis was terminated in 1939 and no further attempt was made to study other infectious diseases, since it was realized that the original belief about the role of epidemics in the decline of animal numbers was based only on the fact that the two were sometimes associated. Although it is to be expected that diseases of various sorts are involved in most declines in numbers, there is no evidence that they are a sufficient condition for the type with which we are concerned.

(*b*) *Predation*

If certain properties are assigned to an imaginary predator and prey, the numbers of predators and prey can be made to fluctuate in a manner superficially similar to fluctuations in nature. This superficial resemblance, however, is the same whether the predator destroys its own food supply, as in the model, or whether the prey disappears first for other reasons. In any study of predator–prey relations it is therefore essential to distinguish between these alternatives. In the case of the vole there is no reason to suppose that predation is sufficient to account for recurrent declines in numbers, since these take place whether predation is light or heavy; nor can predators be responsible for the failure of the animals to grow and sometimes to reproduce, nor for the differential survival of the sexes (6). Similar conclusions were reached by Godfrey (17) and Lockie (23).

When predators are suddenly deprived of their normal food they may perhaps deplete some alternative. In testing this possibility care must be taken to see that any association of events is not fortuitous, especially as short-term fluctuations in the numbers of small rodents are bound to coincide with all sorts of unrelated events. An example of this occurred at Lake Vyrnwy, where foxes have always been plentiful. In 1955 large numbers of rabbits died with myxomatosis. Voles also became scarce; but this had been predicted from a knowledge of their populations in 1954. Furthermore there were other parts of Lake Vyrnwy where populations continued to build up their numbers in spite of any additional attention from the foxes. The disappearance of the rabbits thus had no detectable influence on the population trends of the voles.

(*c*) *Food Supply*

When voles are exceptionally abundant they destroy a great deal of their food and cover; but nobody knows the proportion of occasions on which this happens, as only the abnormal instances receive attention out of several thousands of vole populations that presumably reach temporary abundance every 4 years or so. Outbreaks attended by destruction of the vegetation have thus attracted a disproportionate amount of attention, and writers who base most of their conclusions on such instances run the risks usually associated with biased sampling.

At Lake Vyrnwy it is usual to find local patches of damage to vegetation at times of maximum abundance, but the subsequent scarcity of voles seems to be independent of the state of the vegetation. Thus although serious

damage is sometimes associated with high numbers there are so many instances when it is not that there is no reason to suspect that malnutrition or starvation play any necessary part in the recurrent mortality. Nor are there any signs of emaciation in the animals, whose numbers may decline throughout the growing season of the vegetation, in all types of plant community, in wet and dry areas, and regardless of how few voles may be left to compete for food.

At the time of the marking-recapture study described by Leslie *et al.* (21) a botanic survey was undertaken by Mr. W. E. J. Milton of the Welsh Plant Breeding Station, and Mr. J. Lewis of the Department of Animal Health, Aberystwyth. The data from this survey have not been published but were kindly made available for use at Oxford. Damage to the vegetation was too slight to have caused any general food shortage during 1948–50, a period covering a change from great abundance to great scarcity, but the survey showed that there was an annual shortage of green material. Some areas, for example, provided only 3% green material in March 1949. It is easy to imagine that there might be competition for this seasonally reduced food supply, and that the higher the numbers of the overwintered population the higher their mortality rate from starvation; but no supporting evidence could be found. On the special study area, for example (21, Fig. 1), the verges of the road running through it provided 35–85% green material even at this time of year, and total tillers of 300 per 36 sq. in. Yet the voles living along the road did not grow or survive any better than they did anywhere else at Lake Vyrnwy.

The results of this survey thus confirmed the common-sense impression from other occasions that a general shortage of food is neither a necessary nor a sufficient condition for the recurrent type of mortality, and the findings of Summerhayes (27) that voles did not seriously reduce the total amount of vegetation, in spite of having a pronounced effect on its composition.

(*d*) *Weather*

In order to get some idea of the influence of weather it is helpful to study populations that are fluctuating in opposite directions in neighboring habitats. Let us suppose that some populations decline in a given year, while others nearby do not (Fig. 1, year 0–1). Then, if all were similarly exposed to bad weather, we may conclude that it was not a sufficient mortality factor. This type of evidence was provided at Lake Vyrnwy, where one group of populations declined in 1938 while another group remained abundant until the following year (4), and similar evidence has been obtained since. However, although bad weather alone may not be a sufficient condition for a decline in numbers it may be sufficient in conjunction with some other condition. Random variations in weather may indeed be one of the principal factors affecting populations, and are probably a necessary part of the mechanism that tends to make them fluctuate in phase (4, 6). Leslie (20) has constructed a numerical example to show how this effect may be produced.

(*e*) *Shock Disease*

Determinations were made of the liver glycogen content of wild voles taken simultaneously from declining and expanding populations. The animals were killed in the field, often within an hour of capture, and their livers were put immediately into a hot solution of potassium hydroxide; other voles were kept for a few weeks in isolation before being killed. No evidence was discovered to suggest that any of them had a pathological condition that impaired their ability to store glycogen. Elsewhere (8) I have discussed the claim that a condition of this sort (shock disease) occurs among natural populations of snowshoe hares (*Lepus americanus*) and tried to show that inferences from the laboratory data are not only unjustified, but are actually contradicted by the field evidence. The conclusion of Frank (15, 16) that shock disease occurs in *Microtus arvalis* applies only to animals that had been ill-treated in captivity; the condition has not been shown to explain the natural death rate.

(*f*) *Adrenopituitary Exhaustion*

Christian (9) wrote as follows: "Exhaustion of the adreno-pituitary system resulting from increased stresses inherent in a high population, especially in winter, plus the late winter demands of the reproductive system, due to increased light or other factors, precipitates population-wide death with the symptoms of adrenal insufficiency and hypoglycemic convulsions."

This generalization is partly based on the belief, which I believe to be false (8), that shock disease occurs among snowshoe hares in nature; but the picture of adult mortality given above is not that described by Green and Evans (18), who believed that the decline in numbers could be attributed to a high juvenile death rate, probably during the summer, and continuing even at low population densities. There are no data to suggest that Christian's explanation applies to wild vole populations; nor did Clarke (12) succeed in producing a decline in numbers in his outdoor enclosures, in spite of the fact that his animals were overcrowded, and that intraspecific strife was almost certainly limiting their increase.

More recently Christian (10) has expanded his original suggestions about stress to include an effect on later generations. This he believes to come about through the impaired lactation of stressed females, which occurs fairly readily in the laboratory (6, 11). Indeed there is no difficulty at all in interfering experimentally with reproductive processes; the difficulty comes in producing an effect which corresponds to anything going on in nature. Although Godfrey (17) found some young that were underweight at weaning during a decline, the principal problem is still to account for the disappearance of animals that seem to be perfectly normal when they first enter the active population. Their body weights do not support the idea that they are undernourished, and although there are other ways in which faulty lactation might affect them there is no evidence that it does so in nature.

Nevertheless, while I question the validity of Christian's conclusions about the mechanisms involved, his general point of view (10) has much in common with that discussed in the next section.

An Alternative Hypothesis

The present section restates an hypothesis which, though still not established, has not yet been falsified, and describes the difficulties of getting the evidence that would do so. Some of the facts that have to be taken into account (4–7, 14, 17, 21, 23, 27) are that declines in the numbers of voles can take place even though the environment seems to be favorable; that high population density is not sufficient to start an immediate decline, nor low population density to halt it; that the vast majority of animals die from unknown causes, males more rapidly than females; that the death rate can be greatly reduced by isolating the animals in captivity, and that the adult death rate, as in the snowshoe hare (8, 18), is not abnormally high during the years of maximum abundance. These facts are consistent with the proposition that susceptibility to natural hazards increases among generations descended from animals affected by adverse environmental conditions. In one form or another this idea has been put forward by writers from 1868 onwards (6), and although it cannot be accepted until a mechanism has been discovered, it fits the facts and cannot be refuted at the present time. Three components need to be considered: the original adverse changes in the environment, the resultant physiological changes, and the external factors that affect the subsequent probability of dying, or actually kill the animals.

1. Environmental Changes

As shown above no success was met with in the search for relevant changes in any environmental factors that included food, cover, weather, enemies, disease, etc., but excluded other animals of the same sort. For some purposes it is probably convenient to think of the environment of the population in this limited sense, but it neglects the fact that each individual is surrounded by others that differ from it, and towards which it may react differently from the way they react towards it. "Every individual in a population of animals is part of the environment of other individuals. . . . The population is then understood as a group of individuals each having an environment which resembles those of its neighbors but differs from theirs if only because the environment of an individual includes its neighbors but not itself" (Andrewartha and Birch (2, pp. 13–14)). According to this definition, even a complete knowledge of total numbers and of all factors in the "environment of the population" would give inadequate grounds for prediction, unless it could also be assumed that every individual was identical with every other alive then and at any other time. In many mathematical models this is assumed to be the case, and as a first approximation it is sometimes convenient to think of real populations in terms of total numbers or degree of crowding. The approximation, however, may be no more informative than counting a number of coins without observing their denominations or realizing that currencies may depreciate.

The inadequacy of the idea that population processes can always be related to numbers is clearly shown in experimental work. A given cage space occupied by two hostile voles is a much worse environment for both of them

than the same amount of space occupied by a large family unit; and the environment of a dominant animal will be quite different from that of the animals who spend their lives being chased or keeping out of harm's way. Repeatable results cannot be expected, however carefully conditions are standardized outside the group, if interactions within it are highly variable. In the field it is even less to be expected that numbers at one time will bear any necessary relation to numbers later on, if only quantity is taken into account and quality is neglected.

Thus the change in the environment which is here postulated as a necessary antecedent to a decline in numbers of the vole is not simply an increase in numbers, but a change in the nature and frequency of the interactions, which at the present time we do not know how to observe, let alone quantify. Furthermore, if there is selection in favor of genotypes that are better able to stand these interactions (7) they will themselves produce a new kind of environment about which population density alone can tell us very little.

2. Physiological Changes

Although an unknown blood condition may be associated with them (13), little is known about the supposed physiological changes, and perhaps the most that can be hoped for is to get some measure of their effects. This point can be made more obvious by considering an explanation suggested by Leslie and Ranson (22). These authors showed that a vole population with a fixed age-specific schedule of mortality would tend to decline more rapidly than usual if it entered the non-breeding season with an unusually high proportion of the older animals. If we accept this explanation we also accept the fact of senescence in organisms without trying to explain it, and confine our attention to measuring its demographic effects. The explanation now being offered is the same in principle as that of Leslie and Ranson, the only difference being that the change in the quality of the population is no longer attributed to an increase in the proportion of older animals (4), but to an increase in the proportion of animals that are congenitally less viable. For all that is known at present these changes in constitution may also be too difficult for the ecologist to measure directly, and he may have to study them through their effects on growth rate, survival, reproduction, behavior, and reaction to standard tests of various sorts.

3. External Factors

In contrast to hypotheses according to which the animals die a violent death from epidemics, predators, parasites, climatic catastrophes, or shock disease, no specific causes of death are postulated. Nor for the following reasons is it thought to be profitable to try to discover them. At various times in its life an animal has a number of experiences, the last of which, naturally enough, is followed by death. If death comes through a pure accident, such as drowning, most of the animal's previous experiences will be irrelevant to its chances of survival. In other cases, however, many circumstances in its earlier life are likely to affect its probability of dying

later on: quality and quantity of food, reproduction, psychological factors, chronic disease, weather, and other hazards. In order to understand a particular death rate it may be more important to examine early events of this sort than those immediately associated with death. Local forces of mortality, whatever they happen to be, will be sufficient to kill off a susceptible population, though the rate at which they do so will presumably vary.

Even when an animal dies under observation in the laboratory it is hard to determine the causes of death. It may be thought that the problem is simpler in the wild, where many deaths occur from accidents of a kind that is easier to understand than the so-called deaths from old age. This impression may be based partly on misapprehension about causes of death (24), partly on non-random sampling. Although some captive animals live long enough to become decrepit, most of them die before reaching old age, and under the harsher conditions in nature relatively more individuals may be expected to die unpredictably early deaths. Secondly the majority of corpses of animals dying in nature are never found at all, and those that are may well result from the simpler forms of accident. At present we do not know; but there is no real justification for assuming that the ecologist can expect to explain the shape of a survival curve in nature until he can do so for one in the laboratory.

This argument should not be pressed to the point where no attempt is made to find out when and how the greatest changes occur in mortality rates, for example during bad weather or at the onset of breeding. Such associations are to be anticipated, but they may contribute only part of the explanation and may not occur universally.

We may now review the course of the argument. We first considered the simplest type of explanation, that a single antecedent condition, such as an outbreak of disease, was both necessary and sufficient to bring about a decline in numbers. No such unique condition was discovered. The next most simple explanation might have been that declines occur for different reasons on different occasions, and if we knew only that animals became scarce now and then there might be some excuse for accepting almost anything as a sufficient explanation, e.g. bad weather one time, predation the next, and so on. No such solution can be regarded as satisfactory when there are enough details to show that the phenomenon is no mere reduction in numbers, but an association of fairly specific effects that are unlikely to follow except from fairly specific antecedents. We therefore had to consider a third type of explanation involving two or more factors in combination, and including at least one necessary and specific condition. This necessary condition is thought to be an interaction capable of increasing the susceptibility of certain generations to a variety of non-specific agents, including chronic disease or parasites, further interactions with other animals, and a range of physical factors that the animals normally tolerate. According to this view both the specific and non-specific conditions must be satisfied, a change in susceptibility being insufficient to bring about a decline in the absence of the normal

mortality factors, and the latter alone being insufficient to decimate a normal population, or at least to produce the association of effects that characterize the recurrent type of decline.

It is difficult to refute this interpretation at present, since it states that an unknown kind of interaction produces an unknown change in the average properties of the individuals, whose descendants become more susceptible to unknown and principally local forms of mortality. Part of the difficulty is technical: we seldom see wild voles or find their corpses; they live too long and take up too much space when brought into the laboratory to provide data on longevity and fertility; they have too long a generation time; little is known about their genetics, and so on. Finally, we have not yet learned how to set up experimental populations to give results that are both repeatable and relevant to the problem in nature.

The latter difficulty comes from the fact that wild rodents, even when abundant, live fairly well dispersed. A population of breeding adults must be considered "crowded" at a density of 120 per acre in the best habitats at Lake Vyrnwy, or one vole to 40 sq. yd of ground covered in dense vegetation and interspersed with a network of runways in three dimensions. Elsewhere densities may be still lower (17). Contrary, therefore, to what some authors appear to believe, useful results will not necessarily follow from keeping animals at a density several hundred times that occurring in nature, and at the same time failing to provide a substitute for their runways and cover.

Certain consequences of the hypothesis could be tested by applying experimental methods in the field, and departures from the following predictions would tend to discredit the idea in its present form. (1) If animals are prevented from interacting adversely they should go on increasing until they run out of food. (2) If large enough numbers of animals are continually removed from an expanding population, the survival rate of the remainder should continue to be high; but where an adverse physiological change has occurred no reduction in density should be sufficient to reverse a downward trend. (3) Numbers should continue to increase if animals from an increasing population are successfully transferred to an area from which a declining population has been removed; but numbers should continue to decline if animals from a declining population are transferred to a new area.

Predictions of this kind can so easily go wrong because of unforseen practical difficulties, inadequate knowledge, and faulty logic, that the time and money required to carry them out on voles might not be well spent. Other species, however, might be easier to work with if it is reasonable to believe that the problem in the vole is merely a special case of a far more general phenomenon. We must now examine this possibility.

Relation to Other Ideas

Andrewartha and Birch (2, p. 656) summarize the principal aspect of their views in the form of three curves for each of two habitats, in one of which (area B) climatic catastrophes happen more frequently than in the other,

and reduce the population to lower numbers, from which it recovers more slowly. They say "Taking all three curves into account, one can easily see that the animals would be more numerous, on the average, in area A than in area B." According to this scheme population densities are determined by the severity and frequency of bad weather, and no other factors need be postulated. We may express this point of view as implying that, in instances of this type, variations in weather are both necessary and sufficient to determine population density.

According to Nicholson (26) there is more to it than this, however. He, too, recognizes the importance of the physical factors, but argues that "they act as the tools or *instruments of destruction* used by true reactive factors". (A reactive factor is one that is influenced by changes in population density and influences it in turn.) The really essential point of his argument is that without a dependent variable the proportion of the population destroyed would be unrelated to population density, which therefore would not be "governed". However, since the death rate would be altogether different in the absence of the "instruments of destruction", much semantic difficulty can be avoided by assuming merely that both types of factor are necessary in such cases. Stripped of its surplus terminology, Nicholson's view can, I think, be reduced to the testable proposition that a set of independent variables is a necessary but not a sufficient condition determining a given population density. His other necessary condition, as already stated, is the presence of a reactive factor, and it is here that the trouble arises.

In a definition of considerable importance Nicholson (25) states: "*The action of the controlling factor must be governed by the density of the population controlled*". From this it would seem important to discover factors whose *action* varies with population density; but this is not what Nicholson advises: "Instead we must find which of the factors are influenced, and how readily they are influenced, by changes in the density of the animals". A natural enemy which destroys only 1% of the population would be "wholly responsible for control", whereas climate would not, even if it destroyed 98% of the population, because "its action [is] uninfluenced by the density of the animals". A good many writers have accepted this argument uncritically apparently without recognizing the magnitude of the assumption involved or the almost complete lack of empirical evidence.

The action of a physical factor can be measured only by observing the results of a reaction on the part of the organism, and these results are predictable only if the properties of the organism can be assumed to be constant. In the case of population phenomena we can measure the action of weather only by observing its effects on death rates, birth rates, or other parameters of the population, and it seems unrealistic to assume that the characteristics of the individual animals that make up the population are constant and independent of population density. Similar considerations affect the interpretation of most mortality rates, since even the effects of biotic factors such as parasites can also be explained by changes either in host resistance or in the severity of the factor.

Changes in resistance, systematically related to population density, are entirely consistent with Nicholson's main idea about the regulation of animal numbers; but not with his proposition that the action of the physical factors is independent of population density. This axiom can be abandoned, however, or restated in unambiguous terms, without affecting his main principle; and the main principle of Andrewartha and Birch may also be retained, as their idea that population density is chiefly determined by the action of the physical factors is entirely consistent with this action being governed by some population attribute. Both theories already include enough qualifications to make such a synthesis possible. The vole work suggests that population densities are indeed governed or regulated, but that this is most commonly achieved by the action of the physical factors; and since the action of any factor whatever depends upon the properties of the individuals, it seems a priori improbable that the effects of weather are independent of population density.

It is perhaps worth trying to justify the addition of fresh speculations to a subject already overburdened with them by showing their possible application to one of the many studies in which no evidence was discovered to show that numbers are regulated in the manner predicted a priori (as seems to be the case with all studies that have gone on long enough to rule out mere associations as explanations).

"It is difficult to see what factors may be responsible for regulating populations of *Glossina*. *Unfavourable conditions of climate are presumably independent of density and cannot regulate it* [italics mine]. Competition for blood between individual tsetse flies does not, we believe, take place. We are left to suppose that the "enemies" (using that word broadly) of the fly or the puparium must be responsible for the regulation. But it must be admitted that we have no evidence that any particular enemy becomes more numerous or effective at higher densities of the tsetse population. We have indeed very little knowledge of the causes or mechanisms which prevent an indefinite increase of these insects when external conditions are favourable, but one must certainly suppose that some such factor exists" (3).

This passage shows the remarkable persistence of the fallacy that because climatic factors are themselves unaffected by population density therefore their action is also independent of density (or other parameters). Jackson (19) also failed to realize that the effect of weather on tsetse flies can never be measured in isolation from a system involving individuals whose properties may not be constant. Although he found a long-term statistical relation between saturation deficit and birth and death rates, he could not understand why these parameters often fluctuated in opposite directions in two populations exposed to the same weather. "There is thus little doubt that the short-term fluctuations are governed by something intrinsic in the local populations, which if true is sufficient to put the detailed analysis of the effects of climate forever beyond our grasp." In contrast to this conclusion, the ideas developed in the present paper imply that results such as Jackson's are

exactly what one would hope to find in order to understand the effects of climate. His results are consistent with the view that the two populations were significantly different in their properties; and such properties should be easier to investigate when simultaneous samples of animals can be examined from contrasting populations. Indeed the effects of climate are almost certain to be unpredictable until differences in the properties of the animals can be recognized.

At the present time there is an understandable reluctance to believe that the degree of crowding commonly observed in nature could possibly have profound effects on physiological condition. In the case of the tsetse fly, for example, the maximum population density may be only about 1800 ♂♂ per sq. mile in some places, with 2–3 times as many ♀♀, or about 10 flies per acre (3,19). Nevertheless Buxton (3) was prepared to speculate that tsetse flies might have a form of behavior that regulates their population density through dispersal; and Tinbergen (28) considers that such mechanisms may be fairly universal. As we know so little about behavior it would be wrong to assume that it has no important effects on physiology and genetics, and there is little doubt that individual properties should be taken into account more often than is customary in population studies. Perhaps the most notable exceptions are the work on locust phases (1 and earlier work), and physiological types of tent caterpillar (29).

According to the views given in this final section voles probably exemplify a general law that all species are capable of limiting their own population densities without either destroying the food resources to which they are adapted, or depending upon enemies or climatic accidents to prevent them from doing so. If this is true, self-regulatory mechanisms have presumably been evolved through natural selection, and arguments in support of this view can certainly be advanced. In the present paper, however, the only argument required is the purely methodological one that it is best to start with the fewest and simplest explanations possible, and to add to them only when it is clear that there are fundamental differences between similar phenomena in related species.

To assume that all species are capable of regulating their own numbers is entirely different from believing that all populations are in fact so regulated. In particular the assumption that a self-regulatory mechanism has been evolved by natural selection implies that it has been adapted in relation to a more or less limited range of environmental conditions. In unnatural or atypical situations, therefore, the mechanism will not necessarily prevent abnormal rates of increase or recurrent food crises.

It may be difficult to apply these views to other species until criteria have been established for recognizing instances of self-regulation and distinguishing them from instances of other phenomena. The attempt is nevertheless worth making, for birth rates and death rates are likely to be misinterpreted as long as it is assumed that the properties of the individuals are constant at all population densities.

Acknowledgments

The substance of this paper was given in some talks at the Department of Zoology, University of British Columbia, in April 1959, and I wish to thank Dr. Ian McTaggart Cowan, Dr. James F. Bendell, and other members of the department for helpful discussions. Dr. W. G. Wellington was also kind enough to read and comment on the manuscript. I am especially grateful to Dr. E. W. Fager for some very useful advice about an earlier draft of this paper, and my colleagues at the Bureau of Animal Population have, as usual, made numerous constructive suggestions that have also been incorporated in the present version.

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THE GENUS THELANDROS (NEMATODA:OXYUROIDEA) IN NORTH AMERICAN SALAMANDERS, INCLUDING A DESCRIPTION OF THELANDROS SALAMANDRAE N. SP.[1]

G. A. SCHAD

Abstract

Thelandros salamandrae n. sp. is described. *"Oxyuris" dubia* Lehmann, 1954 *nec* Leidy, a *nomen nudum*, is a synonym of the new species. The insufficiently described *"Oxyuris" dubia* Leidy, 1856 is of uncertain generic affinity and is considered a *nomen dubium*. *"Oxyuris" magnavulvaris* Rankin, 1937, known from females only, is placed in the genus *Thelandros*. All published and new geographical and hostal data concerning the genus *Thelandros* in North American salamanders are tabulated.

Introduction

Thelandros minutus Read and Amrein, 1952 is the only *Thelandros* reported, as such, from a North American amphibian. However, at least two oxyuroids recorded from nearctic urodeles as *Oxyuris*, sensu lato, are referable to the genus *Thelandros*. These are *"Oxyuris" magnavulvaris* Rankin, 1937 and *"Oxyuris" dubia* Lehmann, 1954 *nec* Leidy, 1856. Previous assignment of these forms to a definite genus had not been attempted since their males were unknown. Comparison of my material, which includes males, with one of Lehmann's *O. dubia* and with the description of *O. magnavulvaris* (3) indicates that these may now be placed in the genus *Thelandros*.

The author wishes to thank Dr. Lehmann for the loan of a specimen of *O. dubia* and to express his gratitude to Dr. James D. Anderson for providing him with the several *Aneides lugubris* and *A. flavipunctatus* which yielded part of the material described below.

Thelandros salamandrae n. sp.

(Figs. 1–17; measurements, Table I)

Synonymy

Oxyuris dubia Lehmann, 1954 *nec* Leidy, 1856 (*nomen nudum*).

Diagnosis

Male head with inner circle of two minute papillae per lip, and an outer circle of four large sublateral papillae and two amphids; female with three sublabial projections into mouth opening. Vulva not markedly pedunculate. Male tail with two pairs of preanal papillae and two postanal duplex papillae, both of the latter situated along the ventral mid-line. Preadult larva (? 4th stage) spiny.

[1]Manuscript received September 24, 1959.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.

Description: Thelandros (measurements given in Table I)

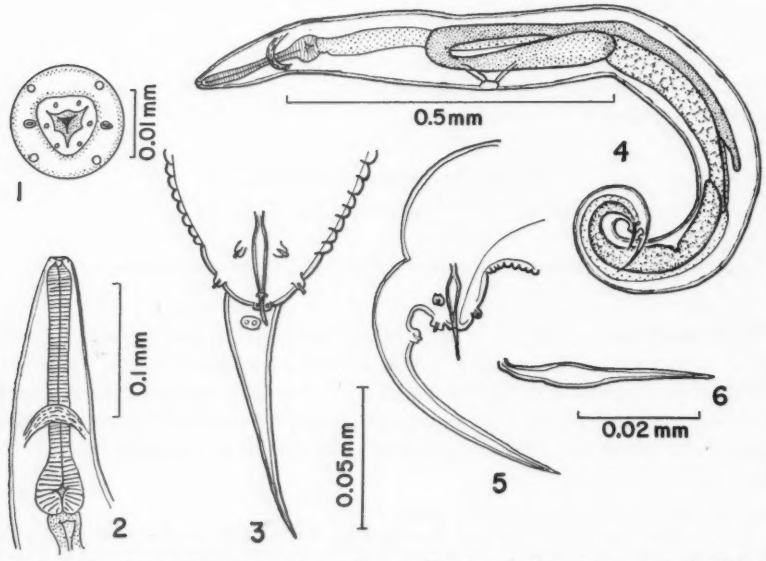
Male.—Mouth bordered by three slightly bilobed lips, each with two minute papillae. Extralabially, six additional cephalic papillae, including the two amphids (Fig. 1). Buccal cavity reduced. Esophagus club-shaped; bulb distinct but not spherical (Figs. 2, 4). Nerve ring posterior to midesophagus. Excretory pore prominent, near anterior third of body (Fig. 4). Tail drawn out into fine point from truncated area at level of cloacal opening (Figs. 3, 4, 5). Two pairs

TABLE I

Comparative measurements of *Thelandros salamandrae* n.sp., *T. minutus*, and *T. magnavulvaris*

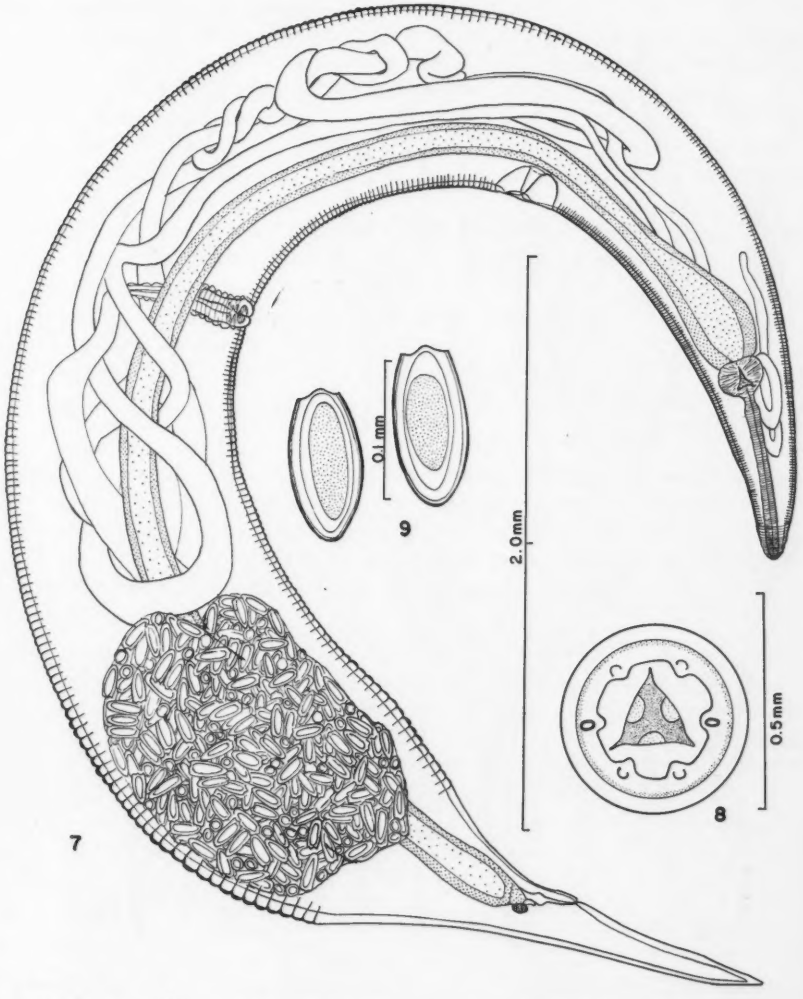
	T. salamandrae		*T. minutus* (after Read and Amrein)		*T. magnavulvaris* (after Rankin)*
Sex	♂	♀	♂	♀	♀
Number measured	11	8	—	—	—
Length	.67–1.52	2.55–7.63	1.490–1.630	4.080–4.680	5.250
Maximum width	.05– .14	.56– .97	.089– .100	.384– .600	.500
Head diameter	.01– .02	.40– .44	—	—	—
Esophagus					
Total length	.14– .20	.54– .75	.204– .231	.388– .600	.530
Bulb width	.03– .06	.12– .16	.049– .059	.083– .119	.110
Bulb length	.03– .05	.10– .13		.079– .092	
Nerve ring from anterior	.07– .12	.10– .17	—	—	—
Excretory pore from anterior	.30– .46	.88–1.87	—	—	—
Tail length	.05– .11	.53– .70	.034– .041	—	.450
Spicule length	.03– .04	—	.040– .056	—	—
Vulva from anterior	—	1.42–3.53	—	—	2.380
Eggs					
Length	—	.12– .13	—	.112– .119	—
Width	—	.05– .07	—	.049– .069	—

*Male of *T. magnavulvaris* is unknown.



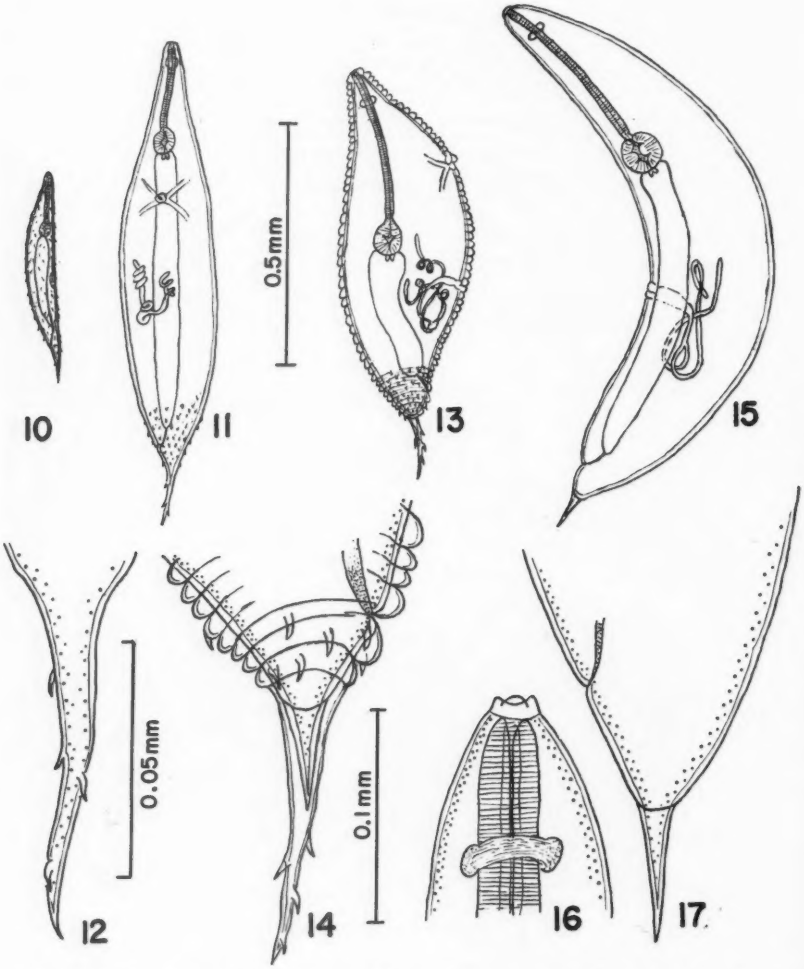
FIGS. 1–6. Male *Thelandros salamandrae*. 1. *En face*. 2. Anterior end. 3. Posterior end, ventral view. 4. Mature male, entire. 5. Posterior end, lateral view. 6. Spicule.

of precloacal papillae; anterior pair subventral, posterior pair lateral (Figs. 3, 5). Two duplex postcloacal papillae situated along midventral line; anterior duplex papilla near apex of genital cone just below cloacal opening. Posterior duplex papilla proximally situated on attenuated tail projection (Fig. 3). Spicule bent proximally, expanded medially, and pointed distally (Fig. 6); sometimes invisible in small males (presumably insufficiently chitinized to permit recognition in young specimens).



FIGS. 7–9. Female and eggs of *Thelandros salamandrae*. 7. Mature female, entire. 8. *En face*. 9. Eggs.

Female.—Body of fully mature individuals characteristic in shape, tapering evenly from small head to maximum width at three-fourths of the body length (Fig. 7). At this level uterus much distended with accumulated eggs. Behind this point body narrows abruptly toward anus. In younger adults edges of body more parallel and without sac-like uterine accumulation of eggs posteriorly. Relatively fine anterior end constant in all observed stages of development (Figs. 7, 10–16). Tail simple and moderately long in mature



FIGS. 10–17. Young stages of female *Thelandros salamandrae*. 10. Young spiny larva (? 4th stage). 11. Late spiny larva (? 4th stage). 12. Tail of same (No. 11). 13. Larva molting to adult stage. 14 Tail of same (No. 13). 15. Young adult. 16. Anterior end of same (No. 15). 17. Tail of same (No. 15).

females (Fig. 7), more spike-like in immatures (Figs. 15, 17). Mouth with three lips. Labial papillae comparable to those of male not observed. Four cephalic papillae and two amphids comparable to external circle of male, present (Fig. 8). Three semicircular projections extend into mouth; one from under each lip. Esophagus slightly constricted just anterior to large bulb. Few ovarian coils extend into esophageal region. Excretory pore, prominent, midway between esophageal bulb and vulva, thus near first quarter of body length. Vulva slightly raised at most and located slightly anterior to mid-body. Oval eggs with operculum at one end (Fig. 9).

Larva (preadult female = ? 4th stage).—Plump, highly contractible, causing marked variation in possible relative positions of organs (compare Figs. 11 and 13). Cuticle spiny (Figs. 10–14); four to seven large tail spines.

Host: *Aneides hardii.*

Habitat: Rectum.

Locality: Six miles northwest of Cloudcroft, Sacramento Mountains, Otero Co., New Mexico.

Type specimens: Holotype male, allotype female; and paratype males, females, and larvae to be deposited in the U. S. National Museum.

Additional material:

Host: *Aneides lugubris* and *Aneides flavipunctatus.*

Habitat: Rectum.

Locality: Rio del Mar, Santa Cruz Co., California.

Specimens: To be deposited in the U. S. National Museum.

Discussion

Of the nematodes reported from North American urodeles three are referable to the genus *Thelandros.* These are *Thelandros magnavulvaris* (Rankin, 1937), *Thelandros minutus* Read and Amrein, 1952, and *"Oxyuris" dubia* Lehmann, 1954 *nec* Leidy, 1856. (Table II lists the hosts, localities, and authors of reports of *Thelandros* spp. in North American salamanders.)

TABLE II

Geographical and hostal distribution of *Thelandros* spp. in North American salamanders

Species	Host	Locality	Author
T. minutus Read and Amrein	*Batrachoseps a. attenuatus*	California	Read and Amrein, 1958
T. magnavulvaris (Rankin) (="*Oxyuris*" *magnavulvaris*)	*Desmognathus f. fuscus*	N. Carolina	Rankin, 1937
	D. ochrophaeus carolinensis	" "	" "
	D. phoca	" "	" "
	D. quadramaculatus	" "	" "
	Eurycea bislineata wilderae	" "	" "
	E. guttolineata	" "	" "
	Plethodon cinereus	" "	" "
	P. glutinosus	" "	" "
	P. yonahlossee	" "	" "
	Diemictylus v. viridescens	" "	" "
	Aneides flavipunctatus	California	Lehmann, 1954
T. salamandrae n.sp. (="*Oxyuris*" *dubia* Lehmann, 1954 *nec* Leidy, 1856)	*Aneides hardii*	New Mexico	Present paper
	A. lugubris	California	Present paper
	A. flavipunctatus	California	Present paper
	A. ferreus	Oregon	Lehmann, 1954
	Ensatina e. eschscholtzii	California	Lehmann, 1954
	Ensatina e. oregonus	Oregon	Lehmann, 1954
	Batrochoseps a. attenuatus	California	Lehmann, 1954

"*Oxyuris*" *dubia* Leidy, 1856 may possibly be conspecific with one of the forms listed above but it was too briefly described to permit recognition. Apparently Leidy's specimens are no longer extant, since they are not included in Walton's revision of the Leidy collections (5). I, therefore, suggest that *O. dubia* Leidy, 1856 be considered a *nomen dubium*.

Lehmann, however, used the name *O. dubia* Leidy, 1856 to designate some female oxyuroids from Pacific Coast salamanders. This was unfortunate since Leidy's description is inadequate; Leidy's material (if it exists) was not examined; the hosts of the parasites differ and the specimens came from opposite sides of the continent. Thus, one does not know whether the two *O. dubia* are conspecific or even congeneric. Lehmann's publication includes no description of his specimens. Therefore, *O. dubia* Lehmann, 1954 must be considered a *nomen nudum*. However, one adult female specimen from Lehmann's collection was studied by me and I conclude that it is conspecific with my material. Thus, *O. dubia* Lehmann, 1954, a *nomen nudum*, is a synonym of *Thelandros salamandrae* n. sp.

Thelandros magnavulvaris and *Thelandros minutus* remain as the only valid North American species of this genus occurring in amphibia, and the new species, *T. salamandrae*, must be distinguished from these. (Table I provides comparative measurements.) *Thelandros magnavulvaris* is known only from females and thus comparisons are limited. However, the following differences are apparent. The markedly prominent vulva characteristic of *T. magnavulvaris* is absent in *T. salamandrae*. The egg of the latter is simpler in that there is no capsule at the antopercular end while such a structure has been described for *T. magnavulvaris*.

T. salamandrae differs from *T. minutus* in the number and distribution of caudal papillae in the male. The new species has two pairs of precloacal papillae followed by two duplex papillae situated along the ventral mid-line. Of the latter, the anterior one is situated just under the cloacal opening, while the posterior duplex papilla is located on the attenuated tail. In *T. minutus* three sets of paired papillae exist, of which two pairs are postcloacal. The subcloacal duplex papilla is not described in *T. minutus*. In the latter the most posterior pair of papillae are inserted laterally at the same level at which one finds the posterior ventral duplex papilla of *T. salamandrae*. Read and Amrein's (4) description of the spicule of *T. minutus* is vague but from the text and figure it is concluded that it is a simple, elongated, pointed shaft. In the new species the proximal end of the spicule is a bent narrow tube which joins a median bulbous portion which in turn leads to the elongate distal point.

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PRODUCTION OF PINE RESIN AND ITS EFFECT ON SURVIVAL OF RHYACIONIA BUOLIANA (SCHIFF.) (LEPIDOPTERA:OLETHREUTIDAE)[1]

P. HARRIS

Abstract

The survival of *Rhyacionia buoliana* (Schiff.) larvae on *Pinus sylvestris* L. is related to the amount of resin encountered during the initial attack on the buds, the larvae being unable to establish themselves in very resinous buds. The resin canals in the buds are developed in response to short days at the end of the summer while the development of the moth is associated with summer temperature. Thus more larvae survive in a warm summer than a cool one as they attack the buds before there is a well-developed resin protection. Also fewer larvae survive on the very resinous *Pinus nigra* Arn. than on *P. sylvestris*. However, the best survival was found on *Pinus contorta* Doug., which also was more resinous than *P. sylvestris*.

Introduction

The pine shoot moth (*Rhyacionia buoliana* (Schiff.)) is particularly serious in parts of North America where the summers are normally warm, and pine stands are frequently so severely ravaged that they are commercially worthless. On the other hand damage is much less serious in western Europe as it is usually possible to remove the damaged trees during thinning, and major outbreaks only occur after the relatively infrequent warm summers. Recent papers by Neugebauer (9) and Voûte and Walenkamp (11) suggest that both the favorability of warm summers and the resistance of different species of pines to the moth are related to the amount of resin in the pine buds at the time of the initial larval attack. Thus possible reasons for the differences in attack are that the European pines are more resistant than are the American species, or that the cool summers in western Europe are less favorable to the moth than the warmer ones of North America. This paper, which is a preliminary to a description of the mortality in a British population of pine shoot moth (in preparation), discusses effects of resin on the life cycle of the moth.

Materials

Three species of pine were used: Corsican pine (*Pinus nigra* Arn.), Scots pine (*P. sylvestris* L.), and lodgepole pine (*P. contorta* Doug.). Corsican pine, a species introduced into Britain from high land in southern and central Europe, is very resistant to attacking *R. buoliana* and rarely suffers serious damage; Scots pine, the native of Britain, is moderately susceptible to attack; and lodgepole pine, a species introduced from western North America, is highly susceptible. Two stands of pine were studied: a 12-year-old mixture of Corsican, Scots, lodgepole pine, and Norway spruce (*Picea abies* L.), near

[1]Manuscript received October 24, 1959.
Contribution from Entomology Research Institute for Biological Control, Research Branch, Canada Department of Agriculture, Belleville, Ontario; from a thesis submitted to the Imperial College of Science and Technology, University of London, in partial fulfilment for the requirements for the degree of Doctor of Philosophy.

Camberly, Berkshire, England, and a 7-year-old Scots, Corsican pine stand on Bagshot Heath. The planting distance in both stands was five feet by five feet.

Review

Many authors consider resin to be the main hazard in the life of the young larva. Voûte and Walenkamp (11), who compared the establishment of the young larvae on a pine tree and on branches that had been cut to prevent resin flow, found that all the larvae on the cut branches established themselves in the buds whereas all the larvae on the tree had disappeared except for one found dead in the needles. This suggests that the young larvae do not persist in attacking very resinous buds, but wander away and, if they do not encounter a less resinous bud, perish. Thus Brookes and Brown (2) suggested that the larvae attack the lateral buds in the autumn because the larger terminal buds are excessively resinous. The larvae can, however, deal with a limited amount of resin by removing it from the mine to the outside of the bud. Neugebauer (9) suggested that the larval saliva assists in coagulating the resin, and Brookes and Brown (2) concluded that the silk with which the larva lines the bud prevents additional resin from oozing into the mine.

Voûte and Walenkamp (11) found that outbreaks of *R. buoliana* in Holland only occurred following a dry July. They suggested that in a wet July the larvae are hindered from establishing themselves by the large amounts of resin forced out of the buds by the high turgor pressure of the cells that surround the resin canals. Conversely, in a dry July the turgor pressure is low and there is little resin to hinder the larvae, so that large numbers survive. Dry weather in July in western Europe usually coincides with hot weather and, in contradiction to Voûte and Walenkamp's explanation, the U.S.D.A. handbook for Naval Stores (1) states that resin flow is more vigorous in hot than in cool weather and that resin flow is not checked by drought until the tree "begins to die".

Basis of Resistance

A species of pine resistant to attack by *R. buoliana* may be either less attractive for oviposition or less suitable for the development of the immature stages than a susceptible species. The number of eggs laid on top whorl shoots was estimated for three species of pines growing together. Two shoots were collected from each of 150–200 neighboring trees in the Camberly stand and examined in the laboratory. The degree of infestation, measured as the average number of eggs and young larvae per hundred shoots, was not significantly different at the 5% level on the three species of pine:

	1956	1957
Scots pine	13.0 ± 2.1 (100 shoots examined)	28.3 ± 4.7 (159 shoots examined)
Corsican pine	14.8 ± 1.1 (88 shoots examined)	20.3 ± 6.7 (123 shoots examined)
Lodgepole pine	16.7 ± 5.5 (108 shoots examined)	43.4 ± 15.6 (106 shoots examined)

Thus, in spite of differences in color, odor, and needle length, the number of eggs laid on each of the three species of pine was similar. However, no eggs were found on 42 Norway spruce shoots examined. These results confirm those of Brookes and Brown (2), who showed that Scots and Corsican pine have a similar number of eggs laid on them and suggested that the basis of resistance is a differential larval mortality.

Larval Survival on Three Species of Pine Growing Together

Population estimates, made through the autumn and winter to measure the decline in the number of larvae per shoot, show that survival was very different on the three species of pine (Table I). Most of this difference arose from the establishment of the third instar larvae in the buds: on Corsican pine very few larvae established themselves, although the survival of the first two needle-feeding instars was good; on Scots pine just over half the larvae survived; and on lodgepole pine most of them survived. When the larvae were in the buds they suffered some mortality from parasites and a fungus disease, but this had little affect on the relative survival on the three pines. These results support the findings of Neugebauer (9) that lodgepole pine suffers heavy damage from *R. buoliana*, Scots pine is less severely damaged, and Corsican pine only rarely damaged.

TABLE I

Number of *R. buoliana* surviving in successive samples on three species of pine growing together

Date	Scots pine		Corsican pine		Lodgepole pine	
	No./shoot	%	No./shoot	%	No./shoot	%
Camberly stand						
Aug. 7–22, 1956	13/100	13.0	13/88	14.8	18/108	16.7
Oct. 20, 1956	13/ 93	14.0	1/78	1.3	10/ 58	17.2
Jan. 14, 1957	4/ 60	6.7	0/62	0	9/ 46	19.6
Feb. 15, 1957	2/ 56	3.6	0/56	0	6/ 56	10.7
May 21, 1957	19/300	6.3	1*	—	21/200	10.5
Bagshot stand						
Aug. 12–15, 1956	27/98	27.6	20/98	20.4		
Sept. 12, 1956	6/36	16.7	4/36	11.1		
Sept. 18, 1956	1/46	2.2	0/46	0		
Jan. 7, 1957	9/82	11.0	1/80	1.3		

*One larva found on 50 trees examined.

The experiment was repeated in 1957 in greater detail on the Bagshot stand by counting the dead as well as the surviving larvae in the samples and by sampling the whole tree instead of only the top whorl. The estimates, representing several trees of each species, are expressed as the average number of larvae per hundred shoots (Table II). They show that (*a*) as in the previous year, there was greater survival on Scots pine than on Corsican pine; and (*b*) survival on both pines was better than in the previous year in that most of the third instar larvae established themselves in the buds on Scots pine and

about half did so on Corsican pine. This greater survival was associated with warmer summer temperatures (Harris, in preparation) and the earlier development of the third instar larvae.

TABLE II

Survival of *R. buoliana* on two species of pine growing together

Date	Larvae per 100 shoots			% dead	% parasitized	No. healthy larvae sampled
	Healthy	Dead	Parasitized			
			Scots pine			
Aug. 27, 1957	7.2	0.12	0.12	1.6	1.6	61
Sept. 6, 1957	5.2	0	0	0	0	43
Sept. 17, 1957	14.6	0	0.49	0	3.3	90
Sept. 30, 1957	10.0	0.42	0.42	3.9	3.9	47
Oct. 22, 1957	9.7	0.80	0.27	7.4	2.5	72
Nov. 21, 1957	7.1	1.51	1.02	15.7	10.6	55
Mar. 6, 1958	6.3	0.31	0.31	4.5	4.5	81
			Corsican pine			
Aug. 27, 1957	7.8	0.56	0	6.7	0	70
Sept. 6, 1957	8.3	0.94	0	10.2	0	53
Sept. 17, 1957	5.0	1.09	0	17.9	0	32
Sept. 30, 1957	4.4	1.13	0	20.4	0	13
Oct. 22, 1957	3.1	0.18	0.18	5.2	5.2	17
Nov. 21, 1957	4.3	0.62	0.31	11.9	5.9	28
Mar. 6, 1958	1.3	0	0.11	0	7.8	12

Parasitism, mainly by the external parasite *Scambus sagax* (Htg.), accounted for a similar percentage of the larvae in the buds on both pine species. Most of the dead larvae found were mummified, by the fungus *Paecilomyces farinosus* (Dicks ex Fr.) Brown and Smith, but a few on Corsican pine were covered with resin which thus may have been the cause of death. Thus the slightly higher percentage of dead larvae on this pine probably reflects the difficulty of larval establishment in the buds. However, the combination of parasitized and dead larvae do not account for all the mortality on Corsican pine. Shallow holes suggested that the missing larvae had attempted to attack the buds and had abandoned them, and then dropped from the tree.

It is concluded that fewest *R. buoliana* survive on Corsican pine because on this tree the larvae have the greatest difficulty in establishing themselves in a bud; more survive on Scots pine because they have less difficulty; and most survive on lodgepole pine as they have little difficulty. On both Scots and Corsican pine the larvae experience less difficulty when they attack early in the summer than when they attack late.

Reaction of the Larvae to Small Amounts of Resin

The larvae can remove small amounts of resin oozing from their mines in the bud. They pick up and manipulate with their mouth parts the small beads of resin oozing from the damaged bud tissue and then spread the resin on the silk tent outside the bud. This was observed several times and the larvae seemed to have no difficulty from resin adhering to their mouth parts.

The presence of small pieces of finely chewed bud, and sometimes a yellowish tinge to the resin lump on the bud, suggested that the larvae might use a secretion to prevent the resin from sticking to the mouth parts.

Small drops of vomitus (obtained by irritating the larvae) with a pH of approximately 8.5 were mixed with resin. The mixture emulsified readily and hardened within a few hours with the formation of leaf-shaped crystals. Untreated resin remained sticky for several days, or even weeks in cool weather. The emulsification of resin from different species of pine was not compared, but it is possible that the susceptibility of a pine to attack is partly related to the ease with which the resin is emulsified.

It is concluded that the larvae can remove small amounts of resin from the bud by using vomitus, or saliva as suggested by Neugebauer (9), to prevent the resin from sticking to its mouth parts.

Reaction of the Larvae to Larger Amounts of Resin

Corsican pine buds damaged on the tree in September exude large amounts of resin. Shoots that are clipped from the tree shortly before the buds are damaged produce smaller amounts, and no resin is produced if the shoots are stored for a day before the bud is damaged. It was found that if a hole simulating a larval mine was made in the side of the bud about 20 minutes after the shoots were clipped, there was a slow, steady flow of resin into it. This was done with 25 buds and, as a control, holes were made in 25 buds that were stored for a day so that no resin was exuded. Third instar larvae were then induced to enter the holes, which they did quite readily when placed on the bud and gently irritated. In the control buds the larvae often sealed the entrance with silk and showed no inclination to leave until the shoots started to shrivel. In the fresh buds the larvae left the holes hurriedly with violent wriggles and contortions after only a minute or so. It was noticed that each of these larvae had a patch of resin on it.

It is concluded that resin is a strong irritant on the larval skin, and causes the larva to abandon the bud. Presumably the irritation acts as a safety mechanism whereby the larva leaves a bud when resin floods into the mine faster than the larva can remove it. This explains why many buds were found with an abandoned mine full of resin (Fig. 3) whereas few larvae were found drowned in resin.

Development of Resin Canals in Pine Buds

The winter bud of *Pinus* is a compound structure with unextended 'telescoped' internodes that contain all the primordia of the following season's growth (10). In the cortical parenchyma, external to the vascular cylinder, there is a ring of longitudinal resin canals that extend from the base to the apex of the bud. These canals can be seen with the naked eye in Corsican pine. There are also small lateral canals that branch from the longitudinal canals and extend to the bases of the bud scales. When a bud is wounded these canals, especially the large longitudinal ones, exude resin.

The development of the longitudinal resin canals in the three species of pine was studied in terminal buds collected throughout the summer from the mixed pine stand at Camberly. The buds were preserved in 70% alcohol, descaled, and then hardened for 15 minutes in absolute alcohol before sectioning. The sections were cut by hand, mounted in glycerine gel, and the area of the bud tissue and the resin canals measured.

It was found that: (*a*) small buds have small resin canals; (*b*) the resin canals in Corsican pine are proportionately larger than those in lodgepole pine, which in turn are larger than those in Scots pine; and (*c*) at the end of July the resin canals taper very abruptly from the base of the bud and do not extend to the bud apex; at the end of August the taper is still marked, but by the end of September the longitudinal canals have widened and extended to within a short distance of the bud apex. For example in five Scots pine buds collected on July 30 the average area of the resin canals halfway up the bud was only 5.4% (range 1.5–11.0) of the canal area at the base of the bud; on August 26 it was 8.4% (range 5.6–15.1); and by September 30 it had increased to 29.7% (range 15.1–46.5) of the area at the base. This is shown diagrammatically in Fig. 1.

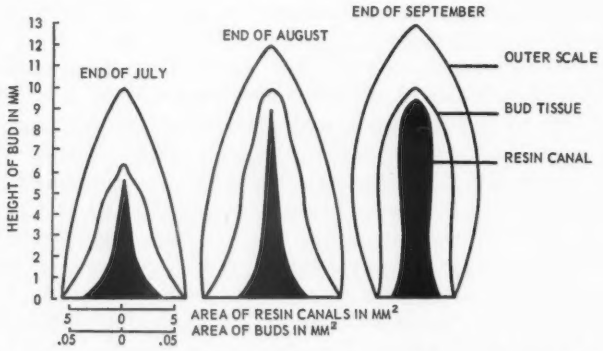

FIG. 1. Diagrammatic illustration of the development of resin canals in Scots pine buds.

Flow of Resin from Damaged Buds

The tops of the terminal buds on the three species of pine in the Camberly stand were cut off and resin was allowed to accumulate for various lengths of time. It was then collected by cutting a thin disc from the top of the truncated bud and dissolving the resin from it with ether. The ether was evaporated at 105° F for an hour and the residue weighed in milligrams. It was found that (around 65° F) the resin gushes out of the bud when it is first wounded but soon ceases to flow: over half the resin exuded had accumulated after only 2½ minutes and flow ceased after 15 minutes.

This method of collecting resin was used to measure the effect of the resin canal taper during July and August on the amount of resin produced. Terminal buds on the three pine species were cut off about a third of the way down

and the resin collected after 15 minutes. The same buds were then cut two-thirds of the way down from the apex and the resin again collected. Two buds were cut on each tree and four trees of each species were sampled periodically through the summer. All the buds were of similar size.

The results in Table III confirm that the resin flow from a bud is related to the size of the canals. Much less resin is produced from the top of the buds in July than later in the summer when the resin canals have enlarged at the apex. Thus, Corsican pine, which has the largest canals, produces roughly three times as much resin as Scots pine, and lodgepole pine, which has the next largest, twice as much. The greater amount of resin exuded from the top of the bud than from the bottom by Corsican and lodgepole pine in September is probably caused by the first cut producing a local and temporary reduction in resin pressure.

TABLE III

Weight of resin exuded by three species of pine bud in 15 minutes (average of eight buds from four trees)

Date	Resin in mg		
	Middle of bud*	Top 1/3 of bud	Bottom 1/3 of bud
		Scots pine	
July 28, 1957		0.4±0.3	7.4±1.6
Aug. 20, 1957		11.5±1.9	17.6±3.2
Aug. 30, 1957		10.3±1.6	18.8±4.5
Sept. 25, 1957		7.4±2.1	6.4±1.7
Feb. 15, 1956	7.4±0.7		
		Corsican pine	
July 28, 1957		3.5±0.3	19.9±4.7
Aug. 20, 1957		5.6±1.3	24.5±5.2
Aug. 30, 1957		10.3±3.8	29.0±5.2
Sept. 25, 1957		28.6±4.6	12.3±1.6
Feb. 15, 1956	20.9±1.8		
		Lodgepole pine	
July 28, 1957		2.0±0.6	18.6±3.9
Aug. 20, 1957		12.8±1.3	15.0±1.8
Aug. 30, 1957		18.4±2.0	14.5±1.0
Sept. 25, 1957		29.0±4.2	13.8±1.8
Feb. 15, 1956	11.7±1.0		

*These figures are for five buds from each tree and five trees of each species.

Lateral buds almost certainly develop simultaneously with the terminal buds (P.F. Wareing, in litt. 1957) and thus as they are smaller they produce less resin. Therefore, by attacking the lateral buds in the autumn the larvae avoid the large amounts of resin, as suggested by Brookes and Brown (2). However, other factors, perhaps the composition of the resin and the ease of emulsification, affect the establishment of the larvae as lodgepole pine is more susceptible to attack than Scots pine despite its larger amount of resin.

Effect of Summer Temperature on Development of Resin Canals in the Bud

Sacher (10) found that the resin canals in *Pinus ponderosa* Dougl. and *P. lambertiana* Dougl. did not differentiate vigorously until bud growth had almost ceased in the autumn, when they widened and lengthened almost to the bud apex. Other authors investigated the stimulus necessary for the cessation of summer growth and the maturation of the resting buds: Krammer (7) found that *P. taeda* L. ceased growing as early in a greenhouse as outside; Jester and Krammer (6) found that *P. banksiana* Lamb. and *P. resinosa* Ait. grew all winter in a long day but not at any time of year in a short day; Wareing (12) found that the onset of dormancy and the formation of the terminal bud in Scots pine seedlings was hastened by short days. Thus from these papers it seems that the maturation of resting buds, and hence the development of the resin canals, depends on day length and is relatively independent of summer temperature.

Effect of Summer Temperature on Development of *R. buoliana*

Unlike the growth of a pine tree, the development of the pine shoot moth is greatly influenced by summer temperature. For example, in Britain adults emerge only 12 days after pupation in a warm summer whereas they may take as long as 5 weeks in a cool one (2). The development of the eggs and of the first two larval instars shows a similar sensitivity. Friend and West (5) found that the egg stage lasted a minimum of 9 days whereas DeGryse (4) reported that it could be twice as long. Brookes and Brown (2) stated that in Britain the first molt normally occurs about three weeks after hatching, but I found that at 77° F molting occurs within 4–5 days of hatching. The second instar is completed within 4–8 days at this temperature. This means that in a warm summer the adults emerge, pair, lay eggs, the eggs hatch, and the larvae develop to the third instar in just over a month, whereas in a cool summer the same development takes about 14 weeks. Thus in a warm summer the third instar larvae attack the buds before they are well protected with resin, whereas in a cool summer the larvae attack after the buds are protected by well-developed resin canals.

Effect of Summer Temperature on the Geographical Distribution of *R. buoliana*

The European distribution of *R. buoliana* reflects the need of a warm summer. Thus, in Britain, the moth is common in southeastern England and is absent from north Scotland. It is most abundant on the Breckland of East Anglia and on the heaths around Aldershot, Berkshire, where the relatively warm summer temperatures are enhanced by the rather bare, sandy soil. In Scotland, *R. buoliana* is replaced by *Coccyx turionana* (Hbn.) (3), which has a similar life history except that it emerges and lays eggs some six weeks earlier. Thus even in the cool summer of Scotland, *C. turionana*

can still develop in time to attack the buds before they are adequately protected with resin. The same type of distribution occurs in Scandinavia: Neugebauer (8) showed that the moth is absent from the littoral region of west Norway, occurs sporadically in the more continental climate towards the Swedish border, and is common in south Sweden.

Damage to Buds in Autumn

The taper of the resin canals in Scots pine affects the damage done to the bud by the third instar larvae in the autumn. Terminal and lateral buds that had been attacked and vacated by the larvae were collected and measured on August 14, 1957. It was found that the entrance hole was usually situated between one-third and one-half way up the bud, so that the larvae avoided the large canals at the base of the bud in their initial attack. In the small buds all the tissue inside the outer scales was eaten, but in the larger buds the larvae ate only the tissue at the top (Fig. 4). The amount of tissue that remained at the base of the bud was proportional to the size of the bud (Fig. 2). This suggests that the larvae eat downward in the bud until the resin canals produce more resin than they can remove. They then leave to attack another bud.

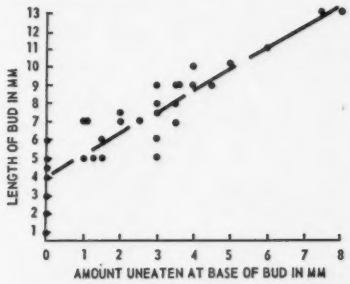

FIG. 2. Relation between bud size and the amount uneaten at the base of the bud in the autumn attack by the third instar larvae on Scots pine.

A similar survey made on Corsican pine showed that only the small buds were attacked successfully. In the larger buds the typical damage was a short tunnel full of resin part way up the side of the bud (Fig. 3). This indicates that the larvae tried to attack the top of the bud but were forced out by the quantity of resin. In 1956, when the larvae attacked the buds in September, many buds on Scots pine also had these partly made, resin-filled holes. In contrast, on lodgepole pine many large buds were attacked and the larvae had continued feeding until either the buds were completely consumed or the remaining tissue was dead. Attacks on lodgepole pine were also characterized by particularly large deposits of resin on the outside of the buds. This suggests that the resin in lodgepole pine is more easily removed, perhaps because it has a different composition, and thus it is not the serious hazard it is in the other pines.

To conclude: Larvae on Scots and Corsican pine can only successfully attack the least resinous buds. This means that on Scots pine the small buds, and the tops of the larger buds early in the summer, are suitable; but on Corsican pine even the small buds may be too resinous. On lodgepole pine all the buds, including the large resinous ones, are successfully attacked. The resin, which is presumably more easily removed, is deposited outside the buds as in the other pines.

Acknowledgments

I wish to thank Professor O. W. Richards and my supervisor Dr. W. F. Jepson of Imperial College, London, for guidance and encouragement.

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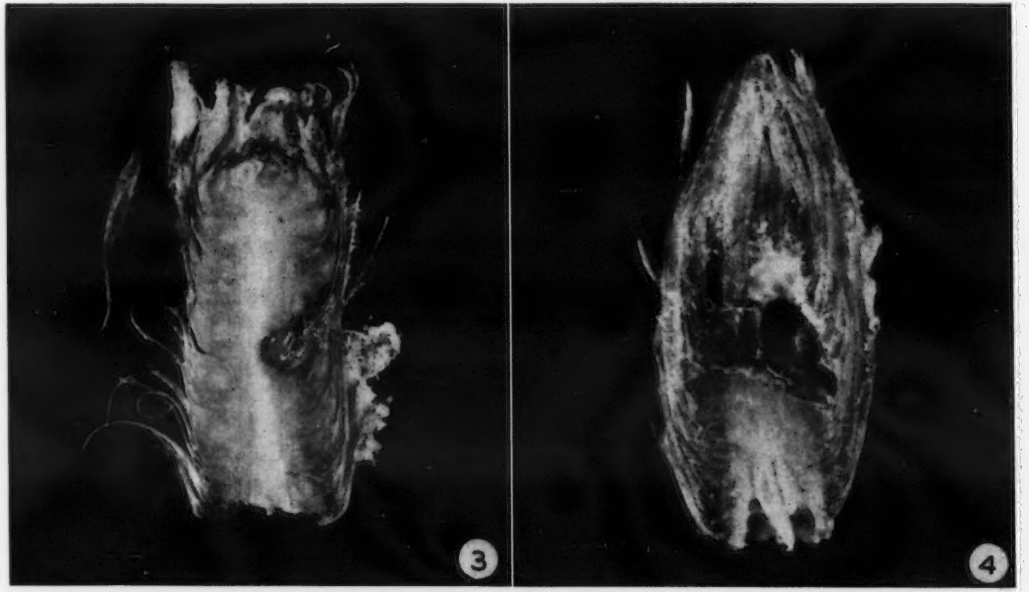

FIG. 3. *R. buoliana* damage in a Corsican pine bud. The larva has stopped mining the bud after making a small hole which has filled with resin.
FIG. 4. *R. buoliana* damage in a Scots pine bud. The larva has consumed the top of the bud inside the outer scales and has left the bottom, which contains the large resin canals, undamaged.

INFLUENCE OF TEMPERATURE, DURATION OF CONDITIONING, AND AGE OF ANAGASTA (EPHESTIA) KÜHNIELLA (ZELL.) (LEPIDOPTERA:PYRALIDIDAE) ON ACCLIMATION TO A SUB-ZERO TEMPERATURE[1]

A. S. ATWAL[2]

Abstract

When *Anagasta* (*Ephestia*) *kühniella* pupae of age groups 0.5, 1.5, 2.5, 3.5, and 4.5 days were conditioned at 5°, 10°, 15°, 20°, and 25° C for 1, 2, 4, 8, and 16 hours, the mortality due to subsequent exposure at −15° C for 4 hours was affected by each of the three factors, age, temperature, and duration of conditioning. As the temperature of conditioning was lowered from 25° to 10° C, the mortality due to the sub-zero exposure gradually decreased; when conditioned at 5° C mortality increased again. When conditioned for 1–16 hours the lowest average mortality was among the pupae conditioned for 4 hours; mortality was highest for 1 and 16 hours. Regardless of the temperature and duration of conditioning, pupal mortality due to sub-zero exposure increased as the age increased.

Pupae conditioned at 10° C, when deacclimated at 25° C for 2–8 hours, exhibited a gradual increase in mortality, showing a tendency to reach the same high level as among the unacclimated.

Adults 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 days old, when exposed to −15° C for various durations, exhibited only a slight difference in the time required to kill 50% (ET_{50}) in each of the age groups; the two oldest groups required the shortest exposures, between 50 and 60 minutes. Moths of a given age group, when conditioned at 10° C, exhibited higher mortality after sub-zero exposure than the controls; hence, there was no acclimation. Without a subsequent sub-zero exposure, conditioning at 10° C had no lethal effect on the insects.

Introduction

Thermal acclimation is well known among marine poikilotherms (6, 8). Bullock (4), in a review, gave many examples of insects that can be acclimated to heat and to cold. However, he agreed with the general belief that insects as a class have poor ability to adapt physiologically to changed environmental temperatures. Dehnel and Segal (7) demonstrated that cockroach nymphs and adults conditioned to cold consumed more oxygen than the controls. Baldwin and House (2) conditioned sawfly larvae to heat and found that the specific gravity of the haemolymph increased. They suggested that changes in the osmotic balance may be concerned. Atmospheric humidity was found to be an important governing factor along with the temperature and duration of conditioning.

The range of lethal low and lethal high temperatures that do not produce sudden death is known to be different for various developmental stages of insects. In the adult stage of a parasitic insect, factors such as starvation and aging may affect acclimation to high temperatures (1). However, some of the factors affecting acclimation to cold are likely to be different; for example, during conditioning at cool temperatures there is little aging.

[1]Manuscript received September 14, 1959.
Contribution from the Science Service Laboratory, Canada Department of Agriculture, Lethbridge, Alberta. This work was made possible through the award of a postdoctorate fellowship by the National Research Council of Canada and the provision of facilities by the Canada Department of Agriculture at the Lethbridge Laboratory.
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This paper is a report on some of the factors affecting acclimation of *Anagasta kühniella* (Zell.) to cold. Pupae of different age groups were conditioned at different cool temperatures for various periods and the influence of these three factors was studied in terms of resistance produced against a given sub-zero exposure. Adults were also conditioned at low temperatures and their reactions were compared with those of the pupae. Of the various stages of development of *A. kühniella* the pupae are known to have the lowest metabolic activity at a given temperature and the adults the highest (9).

Materials and General Methods

The insects were reared on whole wheat flour in glass culture jars 12 in. in diameter in a constant-temperature room maintained at $25 \pm 0.5°$ C. These cultures were started from a general stock of *A. kühniella* kept at 23–27° C for several years. When fully fed the larvae crawled to the inside of the nylon lids. From here they were transferred individually to 3/4-in. cubic boxes of clear plastic to pupate.

As far as possible, pupae of a uniform age were used. In certain cases pupation was observed every hour, but for most of the experiments it was only possible to maintain an accuracy of ± 5 hours. In the latter cases collections were made as follows: At 5.00 p.m. the insects that had pupated during the day were discarded so that those pupating during the night could be collected next morning. At 9.00 a.m. pupae less than 6 hours old, which are lighter in shade than yellowish brown (on pupation they are pale green), were also discarded. Thus the remainder were those that had pupated between 5.00 p.m. and 3.00 a.m. with the mid-point at 10.00 p.m. If the pupae were used at 9.00 o'clock that morning then age was considered to be 0.5 day (± 5 hours). Similarly, if they were used the next day or the day after, their age was considered to be 1.5 days or 2.5 days, and so on.

Often the number of pupae required for one experiment was larger than could be procured in 1 day or 1 week if pupae of many age groups were needed, as in a $5 \times 5 \times 5$ (age of pupa, temperature of conditioning, duration of conditioning) factorial experiment. In this case the pupae procured on 5 consecutive days were used. On the fifth day pupae of each of the age groups were equally distributed among all the 25 treatments. This was repeated until all 125 combinations were based on 20 pupae each. The same procedure was followed for other similar experiments.

When pupae were to be conditioned, they were exposed at a given temperature for the required period and the degree of resistance produced was measured by the number that withstood a 4-hour (45 minutes for moths) exposure at $-15°$ C. The temperature inside the plastic boxes fell to $-15°$ C within 10 minutes. The insects were returned to the rearing temperature (25° C) for the mortality counts. The first experiments revealed that 10° C was optimum for acclimation; therefore, later experiments were conducted at this temperature. When moths emerged from pupae that were exposed at $-15°$ C, some had physical deformities: their wing pads were improperly

opened, their antennae and legs were deformed or missing, and often they could not completely emerge. Where there was no initiation of emergence the moths died inside the pupal cases in advanced stages of development. This showed that the sub-zero exposure did not cause immediate death of the pupae. In fact, a 72-hour exposure at −15° C did not cause freezing in any of 200 pupae, although death must have occurred much earlier. For the calculation of mortalities, active normal moths and active moths with slightly abnormal curled wings more than half open were considered alive, as they were later observed to mate and lay eggs. Sluggish moths with unexpanded wings, half-emerged moths, and those that did not begin emergence were all recorded as dead.

The adults used were collected once a day; thus their age was correct to ±0.5 day. Since it was found that age did not influence their resistance greatly, the accuracy of the age group is considered adequate. On conditioning at 10° C, the only temperature tested, the moths became sluggish but maintained a normal position on their legs. On exposure to −15° C, they lay on their backs within 5 minutes. When returned to 25° C some of them revived gradually; at first they moved their legs and antennae, then fluttered their wings, and after 2–5 hours regained their normal standing position. Many took longer than 4 hours to revive and fell on their backs repeatedly. The dead were counted 24 hours after exposure to −15° C. Even by that time there were always a few individuals that did not keep their position when helped to stand on their legs and in a few seconds again fell on their backs. These were recorded as dead.

Before F or Students' t tests of significance were applied to the experimental data the percentage mortalities were transformed to arcsines. There was 10% natural mortality among the general stock of non-experimental pupae which, no doubt, was also present among those used for various experiments. It has not been taken into account in tabulating the data. None of the untreated moths died within 4 days.

Results and Discussion

I. *Acclimation of Pupae*

Percentage mortalities were determined among pupae 0.5, 1.5, 2.5, 3.5, and 4.5 days old that were conditioned at 5°, 10°, 15°, 20°, and 25° C for 1, 2, 4, 8, and 16 hours. Each treatment was based on 20 pupae. As the experimental samples were from the same culture maintained at the constant rearing temperature of 25° C, the experiment was considered to be a 5×5×5 factorial in a single randomized block and the effects were tested for their linearity or other higher orders. In a similar experiment pupae were conditioned at 5°, 10°, 15°, 20°, and 25° C for 1, 2, 4, and 6 days. The ages of the five groups used were 0.5; 1.5 and 2.5; 3.5; 4.5, 5.5, and 6.5; and 7.5 days. Except for the difference in the duration of conditioning and the age groups used, the experiment was conducted in the same way as described above.

(*a*) *Temperature of Conditioning*

The effect of temperature of conditioning was significant at the 1% level. For conditioning up to 16 hours as the temperature of conditioning was lowered from 25° C to 10° C, the percentage mortality on exposure at −15° C for 4 hours gradually decreased, but it increased again as the temperature was lowered to 5° C, where mortality was about as high as for conditioning at 15° C (Table I). However, for conditioning for 1–6 days, this reversal in mortality did not occur (Table II). When the arcsine mortalities for the shorter periods of conditioning were plotted against the temperature of conditioning the points were closest to a calculated cubic curve (Fig. 1).

TABLE I

Mean mortalities[a] due to a 4-hour exposure to −15° C among pupae of different age groups conditioned at various temperatures for various periods

Temperature of conditioning*		Duration of conditioning*		Age of pupae	
°C	Arcsine (%)	Hours	Arcsine (%)	Days	Arcsine (%)
5	51.1***	1	57.5***	0.5	46.3***
10	46.0	2	54.5	1.5	49.6
15	53.4	4	51.9	2.5	56.7
20	58.9	8	52.9	3.5	59.3
25	65.7	16	58.3	4.5	63.2
Standard error	±1.32		±1.32		±1.32

[a]Based on 500 pupae.
*Interaction between temperature and duration of conditioning significant at 5% level (see text).
***Significant at 0.1% level.

TABLE II

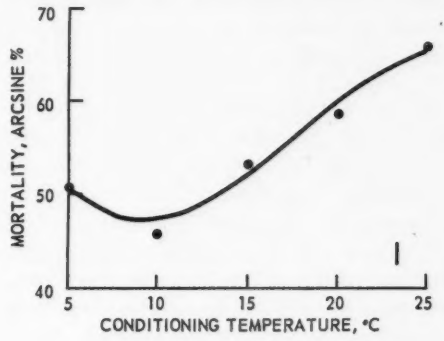
Mean mortalities due to a 4-hour exposure to −15° C among pupae of different age groups conditioned at various temperatures for various periods

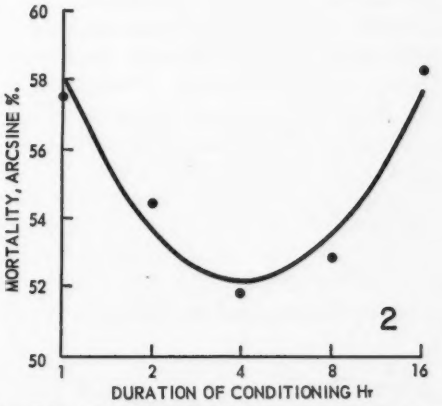
Temperature of conditioning		Duration of conditioning*		Age of pupae	
°C	Arcsine[a] (%)	Days	Arcsine[b] (%)	Days	Arcsine[a] (%)
5	34.7***	1	46.7**	0.5	39.0***
10	36.9	2	45.9	1.5 and 2.5	38.3
15	43.7	4	51.4	3.5	46.2
20	63.0	6	60.2	4.5, 5.5, and 6.5	53.6
25	71.8			7.5	73.2
Standard error	±2.21		±1.97		±2.21

[a, b]Based on 400 and 500 observations, respectively.
*Interaction between temperature and duration of conditioning significant at 5% level (see text).
**Significant at 1.0% level.
***Significant at 0.1% level.

It is indicated from Fig. 1 that mortality was lower among the pupae that were conditioned at 10° C than for those conditioned at 5° C. Colhoun (5) noticed a similar effect of 10° C compared with 15° C when *Blatella germanica* L. was acclimated. As the temperature of conditioning was lowered from 35° to 15° C the temperature at which chill-coma occurred decreased.

However, the chill-coma temperature for insects acclimated at 10° C was the same as that for the insects conditioned at 15° C. It, therefore, seems that as the temperature of acclimation approaches the range of lethal low temperature the rate of production of resistance is slowed down.





FIGS. 1 and 2. Mortalities among *A. kühniella* pupae on exposure to −15° C for 4 hours, pupae of 0.5–4.5 days of age having been conditioned at various temperatures for 1–16 hours (Fig. 1) and at 5°–25° C for various durations (Fig. 2).

(b) Duration of Conditioning

The effect of duration of conditioning was significant at the 1% level. When pupae were conditioned for 1–4 hours mortality due to the sub-zero exposure decreased gradually; on conditioning for 8 hours there was no further decrease, and on conditioning for 16 hours there was a significant increase (Table I). This increase in mortality was confirmed in the second experiment, in which pupae were conditioned for 1, 2, 4, and 6 days; with increase in the duration of conditioning there was a definite over-all increase in percentage mortality (Table II).

The original data showed that for conditioning at 20° and 25° C (control) there was an increase in mortality as the duration of conditioning was increased, which was probably due to aging. However, at the lower temperatures there was little aging.

When the orthogonal components were calculated for their level of significance, the effect due to duration of acclimation for shorter periods (1–16 hours) showed quadratic response; the point of focus was at 4 hours of acclimation (Fig. 2). If the pupae were conditioned for longer or shorter periods, mortality due to the sub-zero exposure increased gradually.

Baldwin and Riordan (3) observed in the parasite *Dahlbominus fuscipennis* Zett. that the maximum resistance by heat acclimation was produced at 36° and 32° C in 2 and 3 hours, respectively. The fall in resistance after the peak was attributed to the effects of starvation and desiccation, the latter probably upsetting the water balance and increasing the specific gravity of haemolymph. Their surmise about the causes of decline in resistance can be questioned on the ground that the time in which resistance rose to the maximum and then declined was probably not enough for these processes to have produced such a sharp decline in resistance, since the experiments were conducted in saturated air. The authors were aware of the difficulties when they pointed out that even with more prolonged treatment at 36° C the tolerance of the insects to heat was greater than at 23° C; in fact, this relationship held for treatment up to 24 hours. It is possible that a decline after the peak of resistance may be a normal trend in physiological acclimation, particularly at abnormally high (36° C and 32° C for *D. fuscipennis*) and abnormally low (10° C for *Blatella* and 5° C for *Anagasta*) temperatures of acclimation.

Within the range of optimum temperatures (from above 5° C to 20° C) of acclimation the maximum resistance is probably produced within a specific time at a certain temperature after which the ceiling of resistance may be maintained within that particular stage of development. Acclimation is not a change that continues indefinitely. Because of the short periods of time involved enzymatic changes may be suspected.

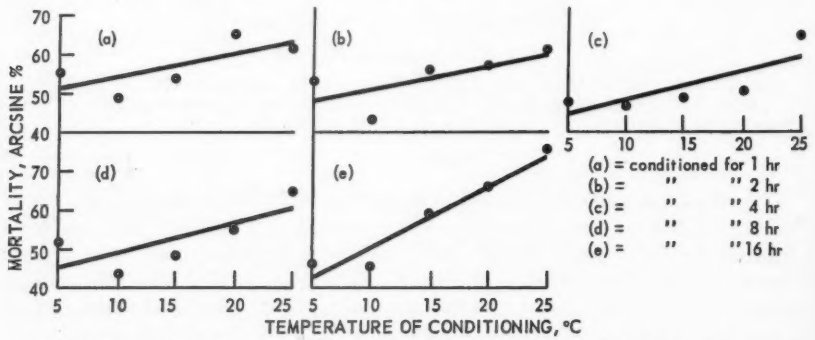


FIG. 3. Mortalities among *A. kühniella* pupae on exposure to −15° C for 4 hours, pupae of 0.5–4.5 days of age having been conditioned at various temperatures for various durations of time.

It would seem that the maximum resistance to cold in *Anagasta* pupae is dependent on the stage of development and the specific temperature and duration of conditioning. This indicates that the processes of physiological conditioning may be associated with the production of a resistance factor which probably fortifies some vital system.

(*c*) *Interaction of Temperature and Duration*

The interaction of the linear response to temperature and duration was significant at the 5% level (Table I). This indicated that the slope due to temperature increased with duration of conditioning. Mortality was generally reduced as the conditioning temperature was lowered (except at 5° C); reduction became progressively more pronounced as the duration increased (Fig. 3). Effects due to other interactions were not significant.

(*d*) *Age of Pupa*

The effect of age of pupa was significant at the 0.1% level. Excluding the effects of temperature and duration of conditioning, mortality among pupae due to sub-zero exposure increased as the age of the pupae increased (Tables I and II). This relationship was essentially linear (Fig. 4, *a*) within the age groups 0.5–4.5 days (Table I) and curvilinear when the entire pupal life (0.5–7.5 days) is taken into consideration (Fig. 4, *b*). The interactions of age with temperature and with duration of conditioning were not significant, indicating that the influence of age was independent of the other factors.

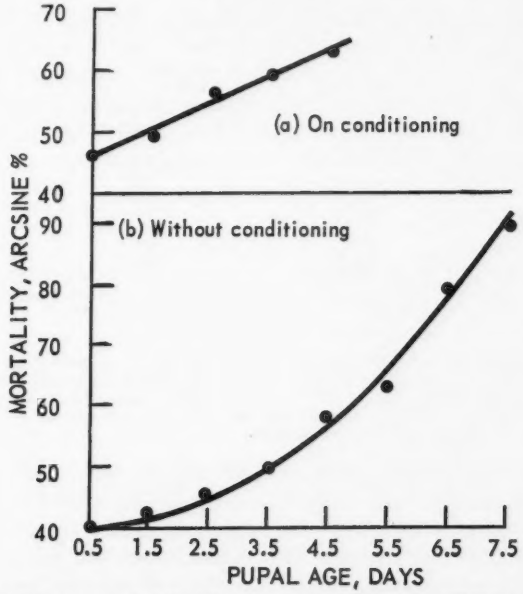


FIG. 4. Mortalities among *A. kühniella* pupae on exposure to −15° C for 4 hours, pupae of 0.5–4.5 days of age having been conditioned at 5°–25° C for 1–16 hours, and pupae of 0.5–7.5 days of age having been exposed without conditioning.

The effect of age of pupa was further investigated in two separate experiments. In the first, pupae in four sets of 20 each of the age groups 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5 days old were exposed at −15° C for 4 hours. An analysis of variance was run as four randomized blocks corresponding to the four sets in each age group. The variance due to blocks was not significant. The effect of age was highly significant at the 0.1% level (Table III). The least significant difference in arcsine per cent mortality between the 0.5-day age group and any other was 15.0.

The general trend was similar to that observed in the factorial experiments described above. With increase in the age of pupae there was a gradual increase in percentage mortality. Compared with the 0.5-day (control) age group, the arcsine percentage mortalities observed in the 1.5-, 2.5-, and 3.5-day age groups were not significantly different (Table III). Mortalities among the remaining age groups were significantly higher. The arcsine percentage mortalities plotted against days of age showed a curvilinear trend (Fig. 4,*b*), that is, the increase in mortality due to aging was greater among the older pupae than among the younger. Thus mortality produced by sub-zero exposure depends on the stage of development. This relation may possibly be traced to specific physiological processes as development takes place. Pupal development was not prevented by the sub-zero exposure, for fully formed moths were found even in those that did not emerge.

TABLE III

Mean mortalities among pupae of different age groups due to a 4-hour exposure to −15° C

Age	Arcsine[a] (%)	Age	Arcsine[b] (%)
Days (±5 hours)		Hours (±0.5 hours)	
0.5 (control)	40.5	4 (control)	40.0
1.5	42.0	8	50.0
2.5	45.0	16	50.0
3.5	50.2	32	50.3
4.5	58.5	64	66.6
5.5	63.2	128	84.6
6.5	79.0		
7.5	90.0		
Standard error	±5.11		±5.18
Analysis of variance: age, $P<0.001$; least significant difference = 15.0; difference due to replications not significant		Analysis of variance: age, $P<0.01$; least significant difference = 16.3; difference due to replications not significant	

[a, b]Means based on 80 and 75 observations, respectively.

In a similar experiment, pupae 4, 8, 16, 32, 64, and 128 hours old (±0.5 hours) were exposed to −15° for 4 hours. Three sets of 25 pupae in each age group were tested in this way and were considered three replications as randomized blocks. The variance due to blocks was not significant, showing that the sample was homogeneous. The effect of age was significant at the 1% level (Table III). The least significant difference between the 4-hour age group and any other was 16.3.

Compared with the 4-hour old pupae, mortalities among 64- to 128-hour-old pupae were significantly higher (Table III). Between the ages of 4 to 32 hours there were no significant differences but with further aging there was a gradual increase in mortality. Advantage was taken of these findings and for subsequent physiological studies pupae of 6–10 hours old were used.

II. *Deacclimation of Pupae*

Since the results given above proved conclusively that physiological acclimation in *Anagasta* pupae was produced within a few hours, it was reasonable to believe that the resistance produced by conditioning could be reversed if the pupae were subjected to conditions adverse to those necessary for acclimation. To study this aspect of the phenomenon an experiment was designed in which pupae of each of the five age groups, 0.5, 1.5, 2.5, 3.5, and 4.5 days, were divided at random into five sets of equal numbers. The first set was taken straight from the rearing temperature of 25° C to −15° C for a 4-hour exposure; this was one control. The other four sets were acclimated at 10° C for 4 hours and one of them (in each age group) was given a 4-hour exposure at −15° C; it served as a second control. The remaining three groups were maintained at 25° C for 2, 4, and 8 hours so that at this temperature the resistance produced on acclimation might be reversed. This was repeated until each of the 25 treatments was based on 70 observations. It formed a 5×5 factorial experiment in a single randomized block. Thus the controls were also considered treatments. Instead of using pupae of five age groups, pupae of any one age group could have been used in a number of replications but the former procedure was used to find whether the speed of deacclimation among the young pupae was different from that among the older pupae.

The analysis of variance showed that the effect due to age was significant at the 5% level, and that due to the treatments was significant at the 1% level (Table IV).

TABLE IV

Arcsine percentage mortalities[a] due to a 4-hour exposure to −15° C among pupae of different age groups not conditioned, conditioned at 10° C for 4 hours, and deacclimated at 25° C for 2, 4, and 8 hours

Age (days)	Not conditioned	Conditioned at 10° C, and then deacclimated at 25° C for:			
		0 hr	2 hr	4 hr	8 hr
0.5	51	29	45	39	48
1.5	53	33	29	38	36
2.5	57	44	46	37	50
3.5	59	37	40	42	48
4.5	67	42	38	47	42
Mean	57.4	37.0	39.6	40.6	44.8
Standard error			±2.20		

Analysis of variance: age, $P<0.05$; treatments, $P<0.01$; least significant difference = 6.6

[a]Mean based on 70 observations.

The least significant difference between the treatment means was 6.6 compared with the mortality among pupae which were exposed to −15° C for 4 hours without conditioning; the mortalities in all other treatments were significantly lower. However, compared with the mortality among pupae given the same sub-zero exposure after conditioning, the pupae which were given this exposure after deacclimation showed a progressive increase in mortality as the time of deacclimation at 25° C was increased. The only significant difference in mortalities was between the second control and the set deacclimated for 8 hours (Table IV). This indicated that the resistance produced on acclimation at 10° C for 4 hours could be reversed on subjection to a higher temperature.

III. *Acclimation of the Moths*

(*a*) *Age on Time for 50% Mortality* (ET_{50}) *at −15°C*

Moths 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 days (±8 hours) old were exposed to −15° C for 20, 30, 40, 50, 60, 70, 80, 90, and 100 minutes. Each treatment was based on 50 moths. Mortalities were plotted against minutes of exposure to −15° C separately for each age group, and the ET_{50} points were obtained from straight lines fitted by eye. The ET_{50} points for the age groups 0.5 days to 4.5 days were found to fall between 70 and 80 minutes and those for the age groups 5.5 and 6.5 between 50 and 60 minutes. When kept individually at 25° C very few of the moths lived for more than 7 days after emerging. Thus, even though no food was given, the aging of the moths produced very little influence on their tolerance to the sub-zero exposure except that the oldest moths (5.5 and 6.5 days old) were slightly less resistant.

(*b*) *Acclimation at 10° C*

Moths were reared at 25° C, and when 1.5 days old (±8 hours) were acclimated at 10° C for 0.5, 1, 2, 4, 8, 16, 32, and 64 hours, and were then exposed to −15° C for 45 minutes. The insects were distributed among the various treatments at random. An equal number serving as controls were given the sub-zero exposure without acclimation. Each treatment was based on 45 observations. The experiment was repeated 5 times, with the same number of observations in each treatment.

The effect due to blocks was not significant, indicating that the samples were homogeneous. The effect due to treatments was significant at the 0.1% level (Table V).

The least significant difference between the mortality in the control and any other mean was 8.2. Thus, compared with the control, the mortalities for all treatments except that conditioned for 64 hours were significantly higher (Table V). This unexpected injury produced by conditioning at 10° C increased for 4 hours, and then for some unknown reason decreased. Within 64 hours the mortality decreased to a level where it was not significantly different from that of the control (Table V). Cold-conditioning was found to be harmful only when followed by exposure to −15° C, because the moths that were brought to 25° C immediately after conditioning did not show any mortality.

TABLE V

Arcsine percentage mortalities due to a 45-minute exposure to −15° C among moths conditioned at 10° C for various lengths of time

	Hours of conditioning									Standard error
	0[a]	0.5	1	2	4	8	16	32	64	
Mortality[b]	22.6	32.8	43.6	41.6	49.0	45.2	39.0	34.4	28.8	±2.86

Analysis of variance: difference due to replications, not significant; treatments, $P<0.001$; least significant difference = 8.2

[a]Control.
[b]Means based on 225 observations.

Acknowledgments

I am grateful to Dr. R. W. Salt, Science Service Laboratory, Lethbridge, for making valuable suggestions throughout this work and to Mr. C. Reimer of the Statistical Laboratory, Ottawa, for advice regarding the statistical analysis.

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INFLUENCE OF TEMPERATURE AND DURATION OF CONDITIONING ON OXYGEN CONSUMPTION AND SPECIFIC GRAVITY OF THE HAEMOLYMPH OF ANAGASTA (EPHESTIA) KÜHNIELLA (ZELL.) (LEPIDOPTERA:PYRALIDIDAE)[1]

A. S. ATWAL[2]

Abstract

Acclimation in *Anagasta kühniella* pupae within the range of normal temperatures was not associated with changes in the rate of oxygen consumption or specific gravity of the haemolymph. Only when the pupae were conditioned at the extreme temperatures of 5° C and 35° C for about 16 and 8 hours, respectively, did their haemolymph become more concentrated. This was not due to desiccation. Thus the theory of osmoregulation does not explain the phenomenon of thermal acclimation satisfactorily.

Introduction

As poikilotherms cannot maintain constant body temperatures they are adapted to cope with most of the environmental changes in temperature. The actual processes by which adaptation takes place are not yet known. However, in some cases the adaptation may be relatively poor (4).

Investigations by various workers have shed some light on the subject. Dehnel and Segal (6) demonstrated in cockroaches that had been acclimated for 1–3 weeks the capacity for compensation in oxygen consumption. There was a direct relationship between increase in oxygen consumption and decrease in acclimation temperature. When *Tribolium* was acclimated to either high or low non-optimal temperatures for 2 months oxygen consumption was significantly depressed below that of the control (7). Baldwin and House (2) found that moderately high temperatures increased the thermal tolerance in two species of sawfly larvae, accompanied by a corresponding increase in the specific gravity and osmotic pressure of the haemolymph. They generalized that acclimation to both heat and cold may be a result of osmoregulation.

A. kühniella is a short-lived insect and the duration of its pupal stage at 25° C is about a week. The maximum resistance to a 4-hour exposure at −15° C was produced within about 4 hours when the pupae of this insect were conditioned at 10° C. The purpose of these studies was to determine whether that resistance was accompanied by corresponding changes in the rate of oxygen consumption or specific gravity of the haemolymph.

[1]Manuscript received September 14, 1959.
Contribution from the Science Service Laboratory, Canada Department of Agriculture, Lethbridge, Alberta. This work was made possible through the award of a postdoctorate fellowship by the National Research Council of Canada and the provision of facilities by the Canada Department of Agriculture at the Lethbridge Laboratory.
[2]Present address: Government Agricultural College, Ludhiana, Punjab, India.

Materials and General Methods

The pupae were reared at 25±0.5° C and were removed from the silken cocoons before using. The general method of handling the pupae was described in an earlier paper (1). There also it was shown that pupae between 4 and 32 hours old were almost equally resistant to a 4-hour exposure at −15° C. The present studies were conducted on pupae 8±2 hours old, unless otherwise stated. Kirk-Stern microrespirometers were used to measure respiration of individual pupae. Only four pupae could be handled at a time, usually from the same treatment. On any one insect at least six observations were taken at 10-minute intervals. The rate of respiration of a pupa was calculated in microliters of oxygen consumed per gram of body weight per hour.

The specific gravity of haemolymph was determined by the gradient column method as modified by Lowry and Hunter (8). Readings were taken 10 minutes after the standard solutions or haemolymph drops were placed in the gradient.

Haemolymph was collected in a fine dropper by puncturing a pupa between the wing pads and squeezing the abdomen. A clean dropper was used each time. The haemolymph collected this way did not contain fat particles or other body tissues. However, when the pupae became older than 48 hours it was not possible to collect clear haemolymph by this method.

As it was found that the specific gravity of the haemolymph was independent of the weight of the pupa, individual weights were not taken. However, abnormally small pupae were discarded. In a preliminary experiment 500 apparently fully fed larvae were caged individually in plastic boxes. A few pupated every day. The specific gravity of the haemolymph of various age groups was measured. Although the last of the larvae pupated 2 weeks after the first, the concentration of their haemolymph remained the same.

The respiration and haemolymph determinations were made at 25° C.

Results and Discussion

I. *Respiration*

A. kühniella pupae were conditioned at 10° C for 2, 4, and 16 hours. They were then returned to 25° C and after half an hour their respiration was measured. Pupae kept as controls were from the same sample but before their respiration was studied they were cooled at 10° C for half an hour and then warmed at 25° C for half an hour. At 10° C their respiration slowed down immediately and when they were returned to 25° C it increased within 5 minutes and reached a constant rate within 10–15 minutes. Therefore half an hour was considered long enough for the respiration rate to reach a constant level. Means for the various treatments were based on 18 observations from each treatment.

The mean oxygen consumption for the control pupae and the pupae acclimated for 2, 4, and 16 hours was 459, 443, 481, and 431 μl. The differences were not significant, measured by Student's method.

II. *Specific Gravity of Haemolymph*

(*a*) *Influence of Age*

The specific gravity of the haemolymph of pupae 1, 2, 4, 8, 16, 24, 36, and 48 hours old was measured in samples of 30 pupae of each age. The means are given in Table I. The pupae were from the same population but not necessarily the same sample. The age of the pupae 1, 2, 4, and 8 hours old was correct within ±0.5 hour and of those 16, 24, 36, and 48 hours old within ±2 hours.

TABLE I

Specific gravity of haemolymph of *A. kühniella* pupae of different age groups[a]

Age (hours ±0.5)	Specific gravity	Standard error ±
1	1.0423***	0.0020
2	1.0378***	0.0031
4	1.0383***	0.0040
8	1.0352	0.0037
16[b]	1.0334	0.0032
24	1.0345	0.0033
32	1.0376***	0.0022
48	1.0386***	0.0039

[a]Mean based on 30 observations.
[b]Control.
***Significant at the 0.1% level.

The specific gravity of the haemolymph of 1-hour-old pupae was 1.0423 and that of the remaining age groups was lighter (Table I). As the pupae grew older their haemolymph became less concentrated and the 16-hour-old pupae showed the lowest concentration with a specific gravity of 1.0334. Beyond that age there was again a gradual increase in the specific gravity of haemolymph up to the age of 48 hours. Clear haemolymph could not be collected from pupae older than 48 hours so further determinations could not be made. Compared with the specific gravity of haemolymph of the 16-hour old pupae, that of pupae 1, 2, 4, 32, and 48 hours old was higher; the differences were significant at the 0.1% level (Table I). In the pupae between 8 and 24 hours old the consistency of the haemolymph did not vary significantly (Table I).

(*b*) *Influence of Sub-zero Exposure*

Pupae were exposed at −15° C for 2, 4, 8, 16, and 48 hours. They were then kept at 25° C for half an hour and the specific gravity of 10 pupae in each treatment was determined. When the pupae were punctured to obtain haemolymph those exposed for 16 and 48 hours appeared to be dead, although not frozen.

The differences between means for the various treatments were not significant except that the concentration of haemolymph of pupae given a 48-hour exposure was significantly higher ($P < 0.001$) than that of the other treatments (Table II).

(*c*) *Influence of Acclimation*

A. kühniella pupae were conditioned at 5°, 10°, and 15° C for 0 (control), 2, 4, 8, 16, 24, 48, and 96 hours. The humidity was not controlled. Similar

pupae were also conditioned at 30° C and 35° C for 2, 4, and 8 hours in saturated air. After conditioning, the insects were kept at 25° C for half an hour and then the specific gravity of their haemolymph was determined. The experiments on cold acclimation and heat acclimation were conducted at different times. In each experiment the pupae used were from the same sample but to obtain 20 observations in each treatment the experiment had to be repeated 4 times. However, at 5° C the means were based on 40 observations. An equal number of control pupae were taken from the same sample and the specific gravity of their haemolymph was determined as soon as the treated pupae were transferred to various temperatures for conditioning. The pupae conditioned at 10°, 15°, and 30° C did not show significant differences so these data are omitted from Table III.

TABLE II

Specific gravity of haemolymph of *A. kühniella* pupae as affected by exposure to −15° C[a]

Hours of exposure at −15° C	Specific gravity	Standard error ±
0	1.0325[c]	0.0021
2[b]	1.0327	0.0046
4	1.0334	0.0021
8	1.0321	0.0018
16	1.0336	0.0018
48	1.0418***	0.0018

[a]Means based on 10 observations.
[b]Control.
[c]Mean based on 500 observations.
***Significant at the 0.1% level.

TABLE III

Specific gravity[a] of haemolymph of *A. kühniella* pupae after thermal acclimation

Hours of acclimation	5° C	S.E. ±	25° C (control)	S.E. ±	35° C	S.E. ±	25° C (control)	S.E. ±
2	1.0328	0.0026	1.0325	0.0019	1.0330	0.0029	1.0335	0.0018
4	1.0329	0.0023	1.0328	0.0018	1.0318	0.0028	1.0311	0.0022
8	1.0328	0.0024	1.0322	0.0021	1.0342***	0.0032	1.0317	0.0024
16	1.0348***	0.0022	1.0326	0.0021	—	—	—	—
24	1.0346***	0.0022	1.0320	0.0019	—	—	—	—
48	1.0367***	0.0036	1.0345	0.0027	—	—	—	—
96	1.0364***	0.0022	1.0325	0.0022	—	—	—	—

[a]Means based on 40 observations for columns 2–5 and 20 observations for columns 6–9.
***Significant at the 0.1% level.
S.E.—Standard error.

When pupae were conditioned at 35° C only the 8-hour group was significantly higher ($P<0.001$) than that of the corresponding control. As there was no change in the weight of the pupae this could not have been due to desiccation.

When pupae were conditioned for 2, 4, and 8 hours at 5° C the concentration of their haemolymph was not affected, but after 8 hours the specific gravity increased significantly (Table III). There was possibly a progressive additional increase.

Further investigations were made to determine whether a similar physiological change was produced at low temperatures other than 5° C. Pupae were conditioned at each of the temperatures 10, 5, 0, −5, −10, and −15° C for

16 hours and their haemolymph was compared to that of the pupae kept at 25° C. The means were based on 15 observations in each treatment. The concentration of haemolymph of pupae conditioned at 5° C was heavier ($P<0.05$) than that of the controls whereas none of the differences at other temperatures were significant (Table IV).

Cold acclimation in *A. kühniella* pupae does not seem to be associated with a change in the rate of oxygen consumption (Section I) or in the specific gravity of the haemolymph. However, acclimation at 5° C for more than 16 hours gave a significant increase in the concentration of haemolymph (Tables III and IV).

TABLE IV

Specific gravity of haemolymph of *A. kühniella* pupae as affected by conditioning at various temperatures for 16 hours[a]

Temperature of conditioning (°C)	Specific gravity	Standard error ±
25°[b]	1.0342	0.0023
10°	1.0354	0.0026
5°	1.0360*	0.0025
0°	1.0351	0.0018
− 5°	1.0352	0.0026
−10°	1.0335	0.0027
−15°	1.0334	0.0021

[a]Means based on 15 observations.
[b]Control.
*Significant at the 5% level.

The increase produced at 5° C within 16 hours was not due to desiccation. It was not produced at 0° C or sub-zero temperatures in 16 hours (Table IV) and thus may be peculiar to temperatures around 5° C. When the pupae were acclimated at 35° C for 8 hours a similar increase in the specific gravity was noticed. This was not due to desiccation. If pupae are kept at this temperature there is no emergence, which indicates that 35° C is harmful.

Baldwin and House (2), extending the theory of Davenport and Castle (5), suggested that acclimation to heat and cold may be a result of osmoregulation. Baldwin and Riordan (3) applied the same hypothesis to explain the decline in resistance of heat-acclimated parasitic insects. This may be an oversimplification. The present studies on *A. kühniella* show that at temperatures where cold acclimation occurs there is no corresponding change in the specific gravity of haemolymph. It is only when the insects are acclimated at 5° C for 16 hours or more or at 35° C for 8 hours that the haemolymph becomes more concentrated. This may be associated with some harmful influence on long exposures to these temperatures outside the optimum range.

Acknowledgment

I am grateful to Dr. R. W. Salt of the Science Service Laboratory, Canada Department of Agriculture, for valuable suggestions given throughout the course of this work and to Mr. C. Reimer of the Statistical Laboratory, Ottawa, for advice regarding the statistical analysis.

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THE MAINTENANCE OF ECHINOCOCCUS MULTILOCULARIS SIBIRICENSIS WITHOUT THE DEFINITIVE HOST[1]

G. LUBINSKY

Abstract

Hydatid cysts of *Echinococcus multilocularis sibiricensis* have been transplanted serially from cotton rat to cotton rat. These cysts have also proved transferable to white mice and gerbils. Hydatid cysts of the fourth transfer produced scoleces and were infective to dogs.

Introduction

The present paper reports the results of an attempt to maintain *Echinococcus multilocularis sibiricensis* in intermediate hosts without passage through the definitive host, that is, as a strain of "secondary" hydatid propagating vegetatively in a series of transfers from rodent to rodent.

Materials and Methods

Cotton rats, *Sigmodon hispidus*, were used as hosts. Cysts of *E. multilocularis sibiricensis* were transplanted from animal to animal by means of intraperitoneal injections. They were aseptically removed from a previously infected cotton rat and rubbed through wire netting (1/16th in. mesh) with the addition of about 10 times their volume of a penicillin solution in normal saline. One or two milliliters of this suspension were injected intraperitoneally with a 1½-in. hypodermic needle (No. 17) into each of the 6 to 12 cotton rats used for the maintenance of the strain. The needle, directed anteriorly, was inserted about half an inch deep into the left lower quarter of the adbomen. The recipient animals were anaesthetized with ether. Transfers were made every 40 to 75 days.

Results

The strain was started from a cotton rat infected with oncospheres of *E. multilocularis sibiricensis*. The animal was killed *in extremis* 72 days after infection and the suspension of its cysts injected intraperitoneally into six cotton rats. The first transfer of the secondary hydatid was made 75 days after the inoculation of the primary hydatid. Subsequent transfers have had to be made at progressively shorter intervals of 60 and 40 days because of a gradual increase in the growth rate.

Twenty-four hours after the inoculation, the injected fragments of cysts can be found in the abdominal cavity predominantly in the omentum major,

[1]Manuscript received November 3, 1959.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada, and from the Department of National Health and Welfare awarded through the Department of Northern Affairs and National Resources, Northwest Territories.

between the stomach, duodenum, and spleen, as well as near the kidneys and, more seldom, between the liver and diaphragm where their further development takes place.

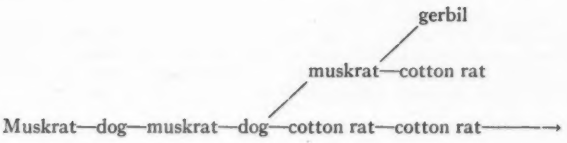
The beginning of the formation of daughter cysts by budding can be observed as early as 48 hours after the injection. A fortnight later, the clusters of cysts may attain 5 mm in diameter; at that age they usually lie free in the abdominal cavity. Although occasionally some free cysts may be found 4 weeks after the transfer, the majority of them at that age are already attached to the peritoneum and at least partially surrounded by the connective tissue of the host, which may be well vascularized.

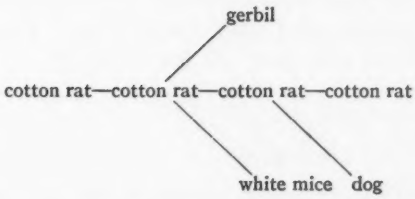
Formation of brood capsules was observed as early as a fortnight after the transfer. Four-week-old clusters of cysts may already contain mature scoleces. Cysts growing in the vicinity of the liver and especially those situated between the liver and diaphragm often cause a partial compression atrophy of the liver and become half impressed in the liver tissue. However, in our material we have also observed an infiltrative penetration of secondary cysts into the tissue of the liver.

At the age of 2 months the transplants form large clusters of minute vesicles containing numerous mature scoleces. These clusters may attain considerable size and their total volume exceed 25 ml. The cotton rats start to die from hydatid disease 6 or 7 weeks after inoculation, and seldom survive for longer than $2\frac{1}{2}$ months. The survival time is inversely proportional to the growth rate of the cysts and therefore gradually decreases with the increase of the number of transfers. As a rule, the cysts develop in all the animals inoculated. Cysts of the fourth transfer proved to be infective for dogs.

A subcutaneous injection of the suspension of ground cysts results in the development of clusters of cysts in the subcutaneous tissue. These may attain a size of 10 by 1 by 1 cm and produce scoleces, although they usually also contain numerous degenerating cysts. The survival time of rats infected subcutaneously is much longer than that of rats infected intraperitoneally. The secondary cysts of the third transfer of this strain maintained in cotton rats have been inoculated successfully into gerbils and white mice. The gerbils proved to be highly susceptible to the secondary hydatid; the transplanted cysts grew fast and attained considerable dimensions. However, in white mice, the percentage of positive results was low, especially when the cysts were inoculated subcutaneously. An attempt to inoculate secondary *E. multilocularis sibiricensis* into guinea pigs by both subcutaneous and intraperitoneal injections has failed.

The history of the strain of secondary hydatid maintained in this Institute can be represented as follows:





The uniform results obtained in experiments with cotton rats suggest that the strain of secondary hydatid maintained in these animals may be suitable for studies on the experimental chemotherapy of hydatid disease.

Further experiments are in progress.

Acknowledgments

I wish to express my thanks to Dr. R. Rausch of the U.S. Arctic Health Research Center, Anchorage, Alaska, for making the strain of *Echinococcus multilocularis sibiricensis* available to the Institute of Parasitology.

MIGRATORY BIRDS AND THE DISPERSAL OF AVIAN MALARIA PARASITES IN THE SOUTH PACIFIC[1]

MARSHALL LAIRD

Abstract

Plasmodium relictum (Grassi and Feletti) is recorded for the first time from the tropical South Pacific (Guadalcanal, British Solomon Islands Protectorate). The hosts—the shining cuckoo, *Chalcites l. lucidus* (Gmelin), and the broad-billed flycatcher, *Myiagra f. ferrocyanea* Ramsay—are new, the former one being a migratory species breeding in New Zealand, but spending the southern winter in the Solomons. The significance of this natural dispersal route for an avian malaria parasite is briefly discussed.

Introduction

In the course of a malaria survey of the interior of Guadalcanal, British Solomon Islands Protectorate, a visit was paid to Betilonga (August 3–4, 1953). This village is situated at an altitude of 1750 ft, some 13 miles south of Honiara, the Protectorate's capital. As a side project, blood films were being collected from as wide a variety of animals as possible. A number of birds were thus shot in this vicinity, and further preparations were obtained thanks to the co-operation of Dr. A. J. Cain and Mr. I. C. J. Galbraith of the University Museum, Oxford, who were using the village as a base for ornithological studies. Two of these birds proved to harbor the avian malaria parasite discussed herein, the descriptive account being based on thin smears of heart blood fixed in absolute methyl alcohol and stained with Giemsa.

Systematic Account

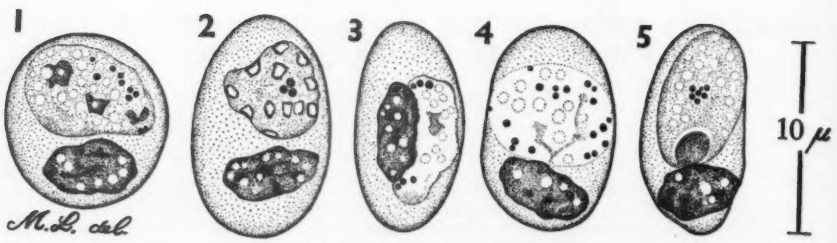
Plasmodium relictum (Grassi and Feletti, 1891) (Figs. 1–5)

Hosts: Chalcites lucidus lucidus (Gmelin) (Cuculiformes: shining cuckoo); *Myiagra ferrocyanea ferrocyanea* Ramsay (Passeriformes: broad-billed flycatcher).

Both infections were light ones, the number of parasitized red cells being below 1 per 10,000. Schizonts (Figs. 1, 2) exhibit up to 12 nuclei, the pigment granules being dot-like, as they are in developing (Fig. 3) and mature (Figs. 4, 5) gametocytes. Microgametocytes (Fig. 4) and macrogametocytes (Fig. 5) are round or oval, and measure from 5.5 to 7.6 μ by from 3.6 to 5.3 μ and from 5.5 to 7.8 μ by from 3.5 to 5.4 μ, respectively (too few were seen to warrant averages). Presence of the parasite is associated with distortion and hypertrophy of the host cell, and marked nuclear displacement.

These data are in agreement with those for *P. relictum* given by various authors from widely separated localities and a diversity of hosts (3, 4, 5, 8).

[1]Manuscript received November 5, 1959.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada. The field work was undertaken during a research project initiated by the Royal New Zealand Air Force and supported by a grant from the N.Z. Department of Scientific and Industrial Research.



FIGS. 1–5. *Plasmodium relictum* (Grassi and Feletti) from *Myiagra f. ferrocyanea* Ramsay (Figs. 1 and 4) and *Chalcites l. lucidus* (Gmelin) (Figs. 2, 3, 5). ×2850

Discussion

Both host records are new, and this constitutes only the second report of an avian malaria parasite from the tropical South Pacific, the earlier record concerning *Plasmodium vaughani* Novy and MacNeal (6). However, *P. relictum* and its variety *spheniscidae* (Fantham and Porter) occur to the south in New Zealand (5), where other species of the genus are thus far unknown from birds.

Doré (2) and Myers (9) suggested that avian parasites brought into New Zealand with introduced birds might have been a factor in the rapid decline in numbers of various indigenous species that took place following European settlement. The author (5), while urging that all livestock imported into the country should be submitted to expert examination for the presence of haematozoa, noted that a much fuller investigation of the blood parasites of both indigenous and exotic birds would have to be undertaken before the hypothesis could be examined critically, and that "no material has yet been examined from any of the migratory birds, which offer the only avenue other than the agency of man for the introduction of avian haematozoa into [New Zealand]". Williams (10) has also drawn attention to the possible significance of migratory species in this connection.

The present findings provide evidence that a natural route for the dispersal of *P. relictum* is indeed open, for one of the hosts, the shining cuckoo, is a winter visitor to the Solomons, commencing to reappear in numbers in New Zealand (where breeding takes place) in August (1). It is submitted that a bird exhibiting parasites in the Solomons in August might be expected to provide a ready reservoir of infection for New Zealand mosquito vectors following the abrupt climatic change and exhaustion (factors favoring relapses) associated with the long southward flight. At the same time, it must not be overlooked that this same host could itself have been a means of introduction of *P. relictum* from New Zealand into the Solomon Islands. The hypothesis of Doré and Myers cannot, therefore, be discarded at this stage.

This is the second illustration of the existence of a natural means of dispersal for avian haematozoa to New Zealand, *Atoxoplasma paddae* (Aragão) occurring there in the silvereye, *Zosterops lateralis* Latham, which originally flew to that country from Australia or Tasmania (5,7).

Acknowledgment

It is my pleasant duty to record my thanks to my wife, my companion on the tramp through Guadalcanal.

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A STUDY OF FILARIA MARTIS GMELIN, 1790 FROM MARTES FOINA AND PEDETES CAFFER[1]

ROY C. ANDERSON

Abstract

A detailed study has been made of specimens of *Filaria martis* from a European mustelid (*Martes foina*) and an African rodent (*Pedetes caffer*). Other species assigned to the genus *Filaria* are considered in the light of this study. Apart from *Filaria martis*, *F. cephalophi* is the only other member of the genus that is satisfactorily characterized. *F. hyracis* is probably a synonym of *F. martis*. The status of *F. carvalhoi* needs clarification but *F. texensis* and *F. conepati* are obviously conspecific with it. The egg and first-stage larva of *F. martis* are described.

Introduction

Although *Filaria martis* has been known for many years, there is still no satisfactory description of it in the literature. This has caused taxonomic problems now that five other species have been assigned to the genus. The author has been fortunate in obtaining specimens of *F. martis* from *Martes foina* (Carnivora) of Italy in addition to specimens from *Pedetes caffer* (Rodentia) of Africa. It is thus possible to redescribe this species in detail and evaluate some of the other species assigned to the genus.

The complex nomenclature and reports of *F. martis* were adequately summarized by Stiles (18). Originally described by Gmelin from specimens from *Martes martes* (Europe), *F. martis* was later reported in *Martes foina* (Europe), *Putorius putorius* (Europe), *Hystrix cristata* (Europe), *Melivora capensis* (Africa), and *Gulo barbatus* (America). The report of *F. martis* in *G. barbatus* is from Molin (9). However, Molin's material came from the thoracic cavity and could not have been *F. martis;* this misidentification probably explains Molin's error in stating that *F. martis* is viviparous. Thus, at the time of Stiles' article, the only valid reports of *F. martis* were from European and African mustelids and the porcupine *Hystrix cristata* of Europe, the latter report going back apparently to Schneider (14).

Stiles (18) and Hall (7) summarized the earliest work on the morphology of *F. martis*. The first more modern description was that of Seurat (16), who reported *F. martis* in *Martes martes* in France. Unfortunately Seurat's excellent description lacked illustrations and this detracted greatly from its value. Monnig (10) described *F. martis* from *Ictonyx capensis* of the Transvaal and gave figures of the head end and a ventral view of the male tail. Yorke and Maplestone (19) gave several highly schematic figures of *F. martis* without indicating the host from which the specimens were derived. Petrov (13) illustrated the head and tail of a female *F. martis* but the author has not been able to obtain this article although the figures are reproduced in Skrjabin and Schikhobalova (17). These descriptions, with the exception of Seurat's,

[1]Manuscript received September 25, 1959.
Contribution from the Department of Parasitology, Ontario Research Foundation, Toronto 5, Ontario.

contain features that are clearly erroneous (e.g. the number and arrangement of the cephalic papillae given by Monnig and the figure of the caudal end of the female given by Yorke and Maplestone which shows the anus terminal in position) and none contains an adequate description of such features as the complex left spicule.

The author gives below a description of specimens from *Martes foina* of Italy. This is followed by a brief description of specimens from the quite unrelated host *Pedetes caffer*, which are believed conspecific with those from *M. foina*. The specimens from *P. caffer* are peculiar in that the protein layer of the egg capsules is absent. This fact has greatly facilitated a study of the early developmental stages and the first-stage larva, certain observations of which are presented below. The latter observations are useful in evaluating the status of *F. texensis* and they indicate for the first time the type of egg and larva found in the Filariinae which are now believed to be primitive filarioids (1).

Materials and Methods

The adult worms were studied in glycerine and lactophenol. The ovum, zygote, and early cleavage stages were obtained from the most posterior ends of the uteri, which are modified to form seminal receptacles. These early stages were examined in lactophenol tinted with cotton blue. The nucleus of the ovum as well as the pronuclei were easily observed in this material. Structures interpreted as the polar bodies were also easily observed although it was not possible to discern clearly the chromosomes in these structures, and attempts to stain by the Feulgen process were unsuccessful presumably because of the age and fixation of the material. Comparable structures have been demonstrated by the Feulgen process as polar bodies in *Diplotriaena thomasi*, however. Fully formed first-stage larvae were studied in both glycerine and tinted lactophenol usually after they had been gently pressed and rolled from their delicate capsules. Many larvae were studied and figured in various positions until a general concept of the morphology was obtained.

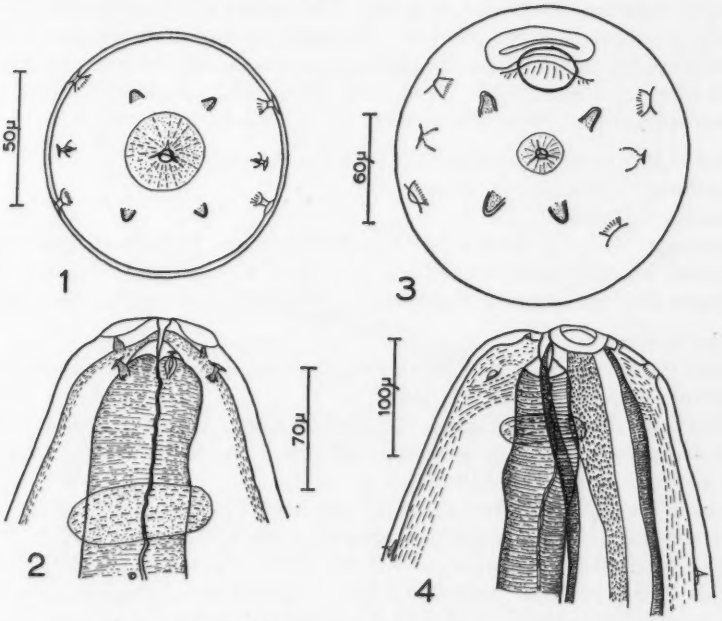
Filaria martis Gmelin 1790

(Figs. 1–2, 4, 7–9, 19–20)

Description

General.—Filarioidea, Filariidae, Filariinae, *Filaria* Mueller, 1787. Long, slender worms tapered at extremities. Anterior end with four pairs of submedian papillae and minute amphids. Buccal cavity well developed, tubular with thin walls, surrounded at base by thick, refractory ring. Oesophagus not surrounding buccal cavity, divided into short, anterior, muscular part and extremely long, glandular part; junction between two parts often obscure. Deirids spinous, close to anterior extremity. Cuticle thick, composed of diagonally running fibers (diagonal to longitudinal axis of body), one group crossing the other at right angles. Flat, inconspicuous, lateral alae extending most of length of body.

Male (four specimens).—Length 60–75 mm. Maximum width near middle of body 0.19–0.26 mm. Muscular part of oesophagus 0.38–0.41 mm, glandular part 6.2–8.2 mm in length, division between the two often indistinct. Nerve ring 0.95–0.12 mm from anterior extremity. Deirids 0.14–0.22 mm from anterior extremity. Excretory pore not discerned. Caudal end somewhat attenuated with two loose coils and long, narrow, caudal alae. Tail 0.13–0.17 mm in length. Caudal papillae as figured. Phasmids not distinguished from papillae. Spicules markedly dissimilar in size and morphology. Right spicule 0.16–0.18 mm in length, curved and tapering like a stout surgical needle. Left spicule 0.56–0.62 mm, consisting of tubular calomus and broad, complicated, membranous lamina.



FIGS. 1–4. *Filaria martis.*
FIG. 1. Anterior end male, *en face* view (*Martes foina*).
FIG. 2. Anterior end male, lateral view (*M. foina*).
FIG. 3. Anterior end female, *en face* view (*Pedetes caffer*).
FIG. 4. Anterior end female, ventral view (*M. foina*).

Female (one specimen).—Length 148 mm. Maximum width near middle of body 0.41 mm. Division of oesophagus not distinct; total oesophagus 13.0 mm in length. Nerve ring obscure, 0.07 mm from anterior extremity. Deirids 0.19 mm from anterior extremity. Excretory pore not discerned. Vulva beside oral opening, surrounded by broad, cuticular ring. Vagina 9.0 mm in length passing into two opisthodelphic uteri one of which finally recurves anteriorly and ends in seminal receptacle in the anterior quarter of body; the other continues posteriorly and its seminal receptacle is in the

posterior quarter of the body. Eggs about 52×36 μ in size consisting of delicate, vitelline membrane, delicate shell (= chorion), and thick laminated, pitted, protein coat. The perivitelline space is generally broad. The chorion is difficult to discern in the fully formed egg but when the eggs were placed in dilute hypochlorite solution the chorion was dissolved considerably more slowly than the protein coat with the result that it was readily distinguished. In some eggs in water the chorion became wrinkled and could be seen lining the inner surface of the protein layer. The chorion is formed early in the development of the egg; the two-celled embryo is enveloped by a hyaline capsule considerably thicker than the vitelline membrane. Eggs at the 30-cell stage have been noted in which the vitelline membrane had already become partially detached from the chorion. The subsequent appearance of the protein layer obscures the chorion. There is considerable variability in the thickness of the protein layer in eggs from the vagina and more anterior parts of the uteri (cf. Figs. 19–20). Tail slightly tapering, 0.51 mm in length with smooth extremity. Phasmids minute, about 45 μ from tip of tail.

Host.—*Martes foina* (Erxleben) (Carnivora, Mustelidae).

Location.—Subcutaneous tissues.

Locality.—Italy.

Specimens.—United States National Museum No. 7376; from the Parona collection, brought to the United States by Prof. C. W. Stiles. Parona's (12) catalogue lists specimens of *F. perforans* (= *F. martis*) from *Mustela foina.*

Other Specimens

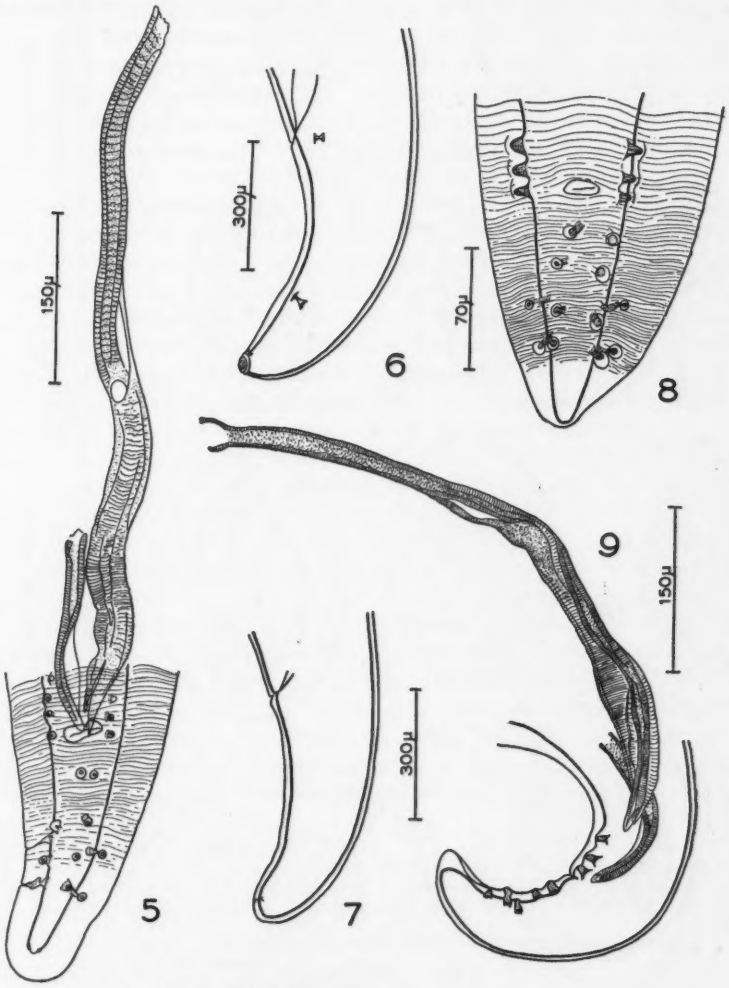
From *Pedetes caffer* (Pallas) (Rodentia, Pedetidae), Africa, British Museum (Natural History), 1.28.39–40. 1958.

Male (one specimen) (Fig. 5).—Length 75 mm. Width 0.37 mm. Muscular oesophagus 0.40 mm, glandular 6.9 mm. Deirids 0.18 mm. Spicules 0.19 and 0.65 mm. Tail 0.16 mm.

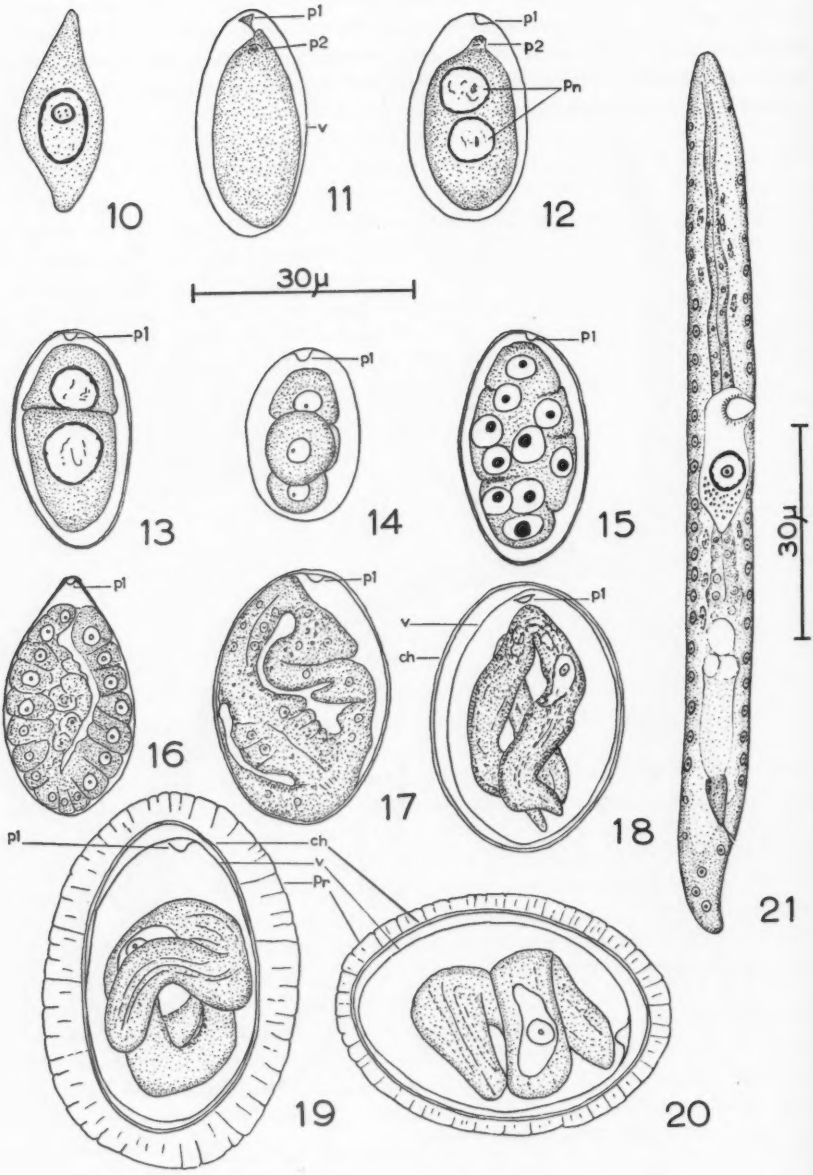
Female (one complete but damaged specimen 190 mm, one anterior fragment 180 mm, and two posterior fragments 220 and 62 mm in length) (Figs. 3, 6).—Width 0.41. Oesophagus 8.9–12.5 mm. Nerve ring 0.11–0.12 mm. Deirids 0.15–0.19 mm. Vagina 8.5 mm. Eggs without protein coats 36×26 μ in size (see below). Tail 0.52–0.77 mm, terminating in rounded plaque provided with numerous tubercles. Phasmids about 30 μ from tip of tail. Postdeirids about 240 μ from tip of tail and a single postdeirid-like structure present on the left side adjacent to anus in complete specimen. In one caudal fragment only a single postdeirid present.

First-stage larva (Fig. 21).—Short, stout, with rounded, unarmed extremities. Length approximately 130–140 μ. Maximum width 10 μ, near middle of body; width at anus 7–8 μ. Oesophagus approximately 80 μ in length, apparently club-shaped, containing numerous nuclei. Nerve ring not discerned. Excretory pore a prominent, transverse slit surrounded by cuticular border, 42–45 μ from anterior extremity. Pore leading into clear vesicle bordered by deeply staining cytoplasm of excretory cell. Excretory cell rich in granular cytoplasm with relatively large nucleus lying about 7 μ

behind pore. Intestine short and inconspicuous, apparently composed of several cells. Rectum about 8 μ in length terminating in prominent anus; cuticle in anal region refractory. Tail bluntly conical about 12–13 μ in length, without spines or terminal protuberances. Elongate, subcuticular nuclei numerous. Buccal cavity, cephalic sensory structures, and genital primordium not discerned.



FIGS. 5–9. *Filaria martis.*
FIG. 5. Caudal end male, ventral view (*Pedetes caffer*).
FIG. 6. Caudal end female, lateral view (*P. caffer*).
FIG. 7. Caudal end female, lateral view (*Martes foina*).
FIG. 8. Caudal end male, ventral view (*M. foina*).
FIG. 9. Caudal end male, ventral view (*M. foina*).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Notes on the Development of the Egg (Figs. 10–18)

Numerous eggs were removed from the seminal receptacles, and various stages in development were studied and interpreted. The ovum is spindle-shaped and contains a large nucleus with a prominent nucleolus (Fig. 10). The earliest developmental stage, presumably after the penetration of the sperm, was surrounded by a delicate membrane interpreted as the vitelline membrane (5). The nucleus had disappeared and refractory bodies, presumably the chromosomes, were noted near the border at one end of the egg (it has been mentioned above that attempts to stain this material by the Feulgen process were unsuccessful). Later the cytoplasm of the ovum shrank from the vitelline membrane and a small portion of the egg was discharged into the perivitelline space at one end of the egg (Fig. 11). This material, believed to be the first polar body, adhered to the vitelline membrane and persisted throughout development, being found on the vitelline membrane of the fully developed egg in the vagina (Figs. 13–18). Unlike the vitelline membrane, it stained intensely blue in cotton blue and lactophenol. Similar structures are found in the eggs of many nematodes (e.g. *Ascaridia*, Physalopteroidea, Diplotriaenidae). Early eggs of *Diplotriaena thomasi* stained by the Feulgen process showed that this structure is composed of chromatic material and it probably represents the first polar body. Later, what has been interpreted as the second polar body was found protruding from the cytoplasm of the ovum on the region of the first polar body (Fig. 12). This rounded elevation, containing refractory bodies (chromosomes?), persisted after the male and female pronuclei had become evident. This second structure was apparently resorbed by the ovum. Fusion of the gametes took place and was followed soon after by the first, unequal cleavage. The egg at this time consisted of two unequal cells lying loosely in a delicate, oval, capsule (Fig. 13). The inner surface of one end of this capsule bore the protuberance regarded as the first polar body. The capsule was probably composed of a shell component as well as the vitelline membrane since another hyaline envelope was found outside the vitelline membrane in the most advanced eggs in the uteri and vagina (Fig. 18); this membrane was interpreted as the true shell or chorion. Both the vitelline membrane and the chorion were delicate and would be difficult to distinguish if in close contact

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FIGS. 10–21. *Filaria martis.*
FIG. 10. Ovum from oviduct.
FIG. 11. Early egg from seminal receptacle showing appearance of first polar body.
FIG. 12. Early egg from seminal receptacle with second polar body and pronuclei.
FIG. 13. Early egg, two-cell stage. Envelope consisting of vitelline membrane and chorion.
FIG. 14. Early egg, four-cell stage. Shell component of capsule probably absent.
FIG. 15. Early egg, 10-cell stage.
FIGS. 16–18. Later stages in development of egg.
FIGS. 19–20. Fully formed eggs from vagina.
FIG. 21. First-stage larva (diagram), lateral view.
FIGS. 10–18, 21. Specimens from *Pedetes caffer*. FIGS. 19–20. Specimens from *Martes foina*.

ABBREVIATIONS: *ch*=chorion; *p1*, *p2*=first and second polar body; *pn*=pronuclei; *pr*=protein layer; *v*=vitelline membrane.

with each other. It was not possible to determine when, during development, the chorion became separated from the vitelline membrane but eggs at the four-cell stage were noted in which the capsule was even more delicate than those of some embryos at the two-cell stage (cf. Figs. 13, 14). The chorion was easily broken and most eggs removed from the uteri were without it. A protein coat was not found on any of the eggs.

Discussion

The specimens from *Pedetes caffer* differ from those of *Martes foina* in that the tail of the female bears a rounded terminal area ornamented with minute tubercles, postdeirids are present, and the egg capsules lack a protein component. The morphology of the terminal end of the female tail is probably variable, however. Seurat (16) and Monnig (10) described the female tail as unornamented whereas Yorke and Maplestone (19) and Petrov (13) figured the tail as ornamented with minute tubercles. Specimens of *Filaria carvalhoi* evidently display the same variability (see below). It is not possible to attach importance to the apparent absence of postdeirids on the specimens from *M. foina* as these structures are often exceedingly difficult to locate and seem highly variable in number and position in filarioids. The absence of protein coats on the eggs is regarded as an abnormality. A few eggs devoid of this component were noted with the normal eggs in the female from *M. foina*. The otherwise striking similarity in the morphology and dimensions of the specimens from the two hosts indicates that they belong to the same species.

Five other species have been attributed to the genus *Filaria*.

(*i*) *Filaria carvalhoi* Freitas and Lent, 1937 (6) from *Conepatus chilensis* of South America. This species is morphologically very similar to *F. martis* but is longer (males 142–160 mm, females 860–880 mm), the eggs larger (112×48 μ), and the oesophagus longer (male 16–17 mm, female 18–21 mm). Other differences mentioned by Freitas and Lent seem less important. The spindle shape of the eggs is clearly the result of distortion in the muscular part of the vagina. However, the distortion of an egg of *F. martis* could not give it the size of the eggs reported for *F. carvalhoi*. Caballero (2), in a study of specimens from *Taxidea taxus berlandieri* of Mexico that he assigned to *F. martis*, regarded *F. carvalhoi* as a synonym of *F. martis*. Caballero's specimens, which certainly seem to agree with *F. martis*, differed mainly from *F. carvalhoi* in that the males were only 56–66 mm, the females 130–153 mm, the eggs 42×23–27 μ in size, and the oesophagus only 9.8–12.5 mm in the male and 11.0–13.2 mm in the female. The caudal end of the female was ornamented with blunt tubercles. The discovery of *F. martis* in a Mexican host suggests strongly that *F. carvalhoi* is a synonym of *F. martis* but it does not follow that the two species ought yet to be regarded as synonyms.

(*ii*) *Filaria hyracis* (Ortlepp, 1937) Chabaud and Rousselot 1956 (11, 3) from *Hyrax* sp. of South Africa. This species, originally described as *Hyracofilaria hyracis*, seems remarkably similar to *F. martis*. The length of the male tail was 0.23–0.26 mm but this rather minor difference can hardly suffice to separate this species from *F. martis*. The illustrations given by Ortlepp agree extremely well with the specimens studied herein. Ortlepp did not see deirids, and the caudal end of the female was unornamented.

(*iii*) *Filaria texensis* Chandler, 1947 (4) from *Mephitis mesomelas varians* of Texas, U.S.A. Chandler noted after comparing his specimens with *F. carvalhoi* that "the Texas specimens conform fairly closely in size with the Brazilian ones; the females vary from 560–780 mm and the single male has a length of 185 mm." It was in the egg that Chandler noted the only significant difference between his material and that from Brazil. He stated that the eggs of the Texas specimens ". . . measure 45–50 μ by 21–23 μ. They have a shell about 1 μ thick with a knob-like thickening at one or both ends and contain a coiled embryo that occupies practically the entire cavity of the egg." A comparison of Chandler's figures and descriptions shows that the eggs were exactly as found in the specimens from *Pedetes caffer*, viz. the protein layer was absent. The discovery in an African and in a North American host of specimens with the same kind of egg but otherwise agreeing with a rather common species in the same general locality indicates that they are not distinct species. *F. texensis* ought, therefore, to be regarded as a synonym of *F. carvalhoi*. Caballero (2) regarded this species as a synonym of *F. martis*.

(*iv*) *Filaria conepati* Schuurmans-Stekhoven, 1951 (15) from *Conepatus suffocans* of South America was distinguished from *F. carvalhoi* in the dimensions of the body and various organs, the morphology of the spicules, and the distribution of the genital papillae. Unfortunately, the description is only of generic value (the only dimensions given are the body length, female 470 mm, male 100–135 mm). Considering the host and geographic distribution it is undoubtedly a synonym of *F. carvalhoi*. Schuurmans-Stekhoven, contrary to Freitas and Lent (6), described the female tail as ornamented with tubercles indicating the same variability in this species as in *F. martis*.

(*v*) *Filaria cephalophi* Chabaud and Rousselot 1956 (3) from *Cephalophus dorsalis castaneus* of North Africa is distinct in that the buccal cavity is thick-walled without a basal ring at its base and the oesophagus is usually long. The male is unknown.

In summary, only two species of *Filaria* are satisfactorily characterized namely *F. martis* and *F. cephalophi*. *F. hyracis* is probably a synonym of *F. martis*. The status of *F. carvalhoi* needs clarification as it is perhaps a synonym of *F. martis* as suggested by Caballero (2). *F. texensis* and *F. conepati* are obviously synonyms of *F. carvalhoi*.

Reduction in the strength of the egg capsule was probably an essential step in the evolution of filarioids since, to invade tissues, the larva would

have had to escape completely from the egg envelope or stretch it to form a sheath about its body. The envelope surrounding the microfilariae of onchocercids seems homologous to the vitelline membrane of *Filaria* and it can probably be concluded that the shell and protein coat of the ancestors of these forms have disappeared during the course of evolution. The vitelline membrane is generally stretched to form a sheath about the body of the microfilaria (cf. McFadzean and Smiles (8)). In the Filariinae the chorion or shell is already exceedingly delicate and any reduction in the strength of the egg capsule would mainly involve the thick protein coat.

The fact that the larva of the Filariinae is fully differentiated (viz. with oesophagus, intestine, rectum, etc.) supports the suggestion (1) that the microfilarioid condition appeared in the course of evolution, only after the larvae of filarioids commenced to invade tissues. A detailed study of the larvae found in the Stephanofilariinae may prove illuminating. The larva of *Filaria* is different from those of the Diplotriaenidae in the apparent lack of spines or cephalic, cuticular ornamentation. Moreover, the larvae of the Aproctinae and the Desmidocercidae differ in having attenuated, pointed caudal extremities.

Acknowledgments

I am grateful to Dr. Grant Inglis and the Trustees of the British Museum (Natural History) for the opportunity to study specimens from *P. caffer*. I am also grateful to Dr. Allen McIntosh of the Agricultural Research Center, Beltsville, Maryland, for the loan of specimens from *M. foina*. Mrs. M. B. Chitwood kindly reviewed an earlier manuscript and suggested that I study material from Europe and I acknowledge my gratitude for her helpful suggestions.

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THE EFFECT OF TEMPERATURE ON THE JUVENILE SOCKEYE SALMON RETINA[1]

M. A. ALI[2]

Abstract

A histological study of the retina of underyearling sockeye salmon, *Oncorhynchus nerka*, reveals that temperature has no effect on the cones either in light or in dark. In light, the retinal pigment is not influenced by temperature, while in the dark, temperature above 15° C brings about a slight expansion.

Introduction

The structure of the *Oncorhynchus* retina and its response to various light conditions have been studied (1, 2, 4). Ali (1) suggested that the state of the retina plays an important role in the downstream migration of the Pacific salmon and the results of a recent investigation support this hypothesis (2).

Available evidence indicates that temperature affects the downstream migration of Pacific salmon fry (6). Temperature is also known to bring about certain positional changes in the retinae of fishes such as *Ameiurus*, *Fundulus*, *Abramis*, and *Carassius* (3). The present investigation was undertaken to study the influence of temperature upon the retina of *Oncorhynchus nerka*. If there is any effect, some correlation between temperature, state of the retina, and migratory behavior might be discernible.

Material and Methods

Hatchery-reared sockeye fry (*Oncorhynchus nerka*) were used in this investigation. These were reared from a group of eggs that were from the Cultus lake spawning grounds, taken on November 16, 1957. The experiments were conducted during the latter part of April, 1958, and the fry had been fed since the second week of March, 1958. They were fed twice daily on a mixture of Clark's dry food (kindly donated by Mr. J. R. Clark of Salt Lake City, Utah, U.S.A.), canned salmon, Pablum (Meade Johnson, prepared mixed cereal), cod liver oil, and yeast extract. The fry used in the experiments ranged from 3.5 cm to 4.4 cm in length.

The experiments were carried out in a light-proof room. Samples were made after exposure to light (400 ft-c) and darkness for an hour and a half at 3°, 10°, 15°, and 23° C. Five fish were sampled from each situation.

Animals were fixed in Bouin's fixative and the routine haematoxylin and eosin method was used. The method of measurement of retinal elements was the same as that used in an earlier investigation (1). Every individual point in the graph (Fig. 1) represents the mean of 50 measurements made, 5 from each of 10 eyes.

[1]Manuscript received October 31, 1959.
Contribution from the Institute of Fisheries and Department of Zoology, The University of British Columbia. This paper embodies part of results obtained during a postdoctoral investigation conducted under the auspices of the Fraser River Hydro and Fisheries Advisory Committee of the University of British Columbia.
[2]Present address: Memorial University, St. John's, Nfld.

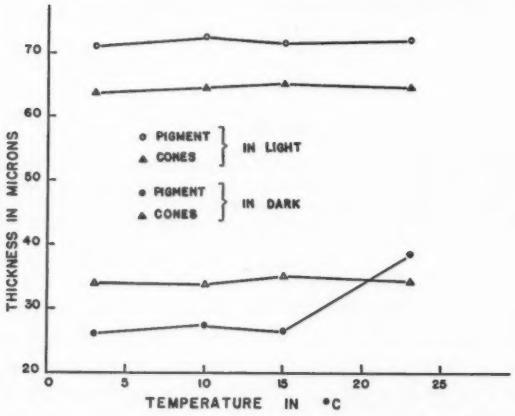

FIG. 1. Graph showing the thicknesses of the cone and retinal pigment layers of *Oncorhynchus nerka* fry in light and dark on subjection to different temperatures.

Results

Influence of Temperature in Light (*Fig. 1*)

Histological examination of the light-adapted retinae of samples taken at 3°, 10°, 15°, and 23° C did not reveal any differences among themselves. The thickness of the cone and pigment layers showed no statistically significant differences (Fig. 1). They presented the appearance of being in a fully light-adapted state. In other words, under constant light, temperature had no influence on the retinal responses.

In comparison, the retinal pigment layers of *Ameiurus*, *Fundulus*, *Abramis*, and *Carassius* have been observed to expand in low as well as in high temperatures, under light (3). The cones and rods, on the other hand, were not affected by temperature at all in the presence of light. It is noteworthy that the results obtained by various investigators (5, 7) using the frog, agree better with those of the present investigation.

Influence of Temperature in Dark (*Fig. 1*)

The dark-adapted cone layers of samples taken from the different temperatures do not differ from one another histologically. Comparison of the thicknesses of these layers from the different samples reveals no statistically significant difference.

The pigment layers of dark-adapted *O. nerka* sampled from 3°, 10°, and 15° C neither showed any histological differences nor did they show any statistically significant differences among their thicknesses. The pigment layers of samples taken from 23° C, however, were somewhat expanded (Fig. 1) and presented the appearance of being in a semiadapted state. The differences in the thicknesses of the pigment layers of samples taken at 23° C and those taken at 3°, 10°, and 15° C were statistically significant at the 0.01 level of significance.

Arey (3) has reported that in the dark, the myoids of the visual cells of *Ameiurus*, *Fundulus*, *Abramis*, and *Carassius* shorten at lower and elongate at higher temperatures, while the retinal pigment expands at lower as well as at higher temperatures. Similar results were obtained by various workers in the case of the frog also (5, 7). Only in the case of *Necturus* (3) did temperature not influence the retinal pigment in the dark.

Acknowledgments

The author's thanks are due Dr. W. A. Clemens, Dr. W. S. Hoar, Dr. N. J. Berrill, and Dr. W. E. Ricker for the criticism of this manuscript. Mr. P. Gilhausen of the International Pacific Salmon Fisheries Commission kindly collected and supplied the sockeye eggs needed for this study.

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THE EFFECT OF VISIBLE LIGHT ON THE OVOTESTIS OF THE SLUG DEROCERAS RETICULATUM (MÜLLER)[1]

NANCY E. HENDERSON AND D. PELLUET

Abstract

Slugs were exposed to light from daylight bulbs combined to give intensities ranging from 50 m-c to 5500 m-c. At 1000 m-c for 10 hours daily for 5 weeks, the cells of the gonad showed changes, first in the germinal epithelium which thickened and produced many nurse cells, then in a general acceleration of meiosis in the male germ cells, resulting in an untidy appearance of the gonad as a whole. By an increase in either the duration or the intensity of the light, further changes are induced, which interfere with cytokinesis in the spermatocytes, yielding large numbers of multinucleate spermatids. Slugs kept for 24 hours in the light at 1000 m-c, or in total darkness, for 5 weeks, show marked changes, but not of the same kind. Darkness induced an increased number of nurse cells to be proliferated from the germinal epithelium, with no effect on cytokinesis, whereas cytokinesis was upset when the animals were kept in the light throughout the night and day. Animals in which these changes had been induced showed a normal ovotestis after being returned to their usual environment.

Introduction

The factors responsible for controlling the differentiation of nurse cells and germ cells of both sexes from the germinal epithelium lining the ovotestis of pulmonate gasteropods are not known. It is possible to induce changes in the individual cells, in addition to accelerating the rate of maturation of the male germ cells, by exposing slugs to low temperatures. Bridgeford and Pelluet (1) suggested from their results with low temperatures that the altered conditions might interfere with a possible enzyme or hormone system in the animal which was responsible for guiding the differentiation of the germinal epithelium into one of three pathways. Other changes in the environment might also be expected to act on this supposed system; the present work is concerned with the effects produced on the cells of the ovotestis, by exposing these nocturnal animals to variations in duration and intensity of light.

No attempt has been made to investigate a possible photoperiodic response in these animals; it is obvious, however, that the induced changes could be ascribed to alterations in the length of the dark period, which accompany changes in the length of the light period.

Material

The histology of the gonad of *Deroceras reticulatum* has been described by Ord and Watts (2) and the cytological details of the cells during development of the ovotestis have been worked out by Pelluet and Watts (3). Spermatogenesis in the five species of slugs found in Nova Scotia are rather similar and the detailed account of maturation of the male germ cells in *Arion subfuscus* given by Watts (4) is so similar to that of *D. reticulatum* that it is unnecessary to repeat it here.

[1]Manuscript received August 14, 1959.
Contribution from the Zoological Laboratory, Dalhousie University, Halifax, N.S.

In comparing the normal young ovotestis with that of the experimentally treated animals, it is necessary to emphasize three outstanding characteristics of the normal ovotestis:

(*a*) The germinal epithelium is a thin layer of cells with elliptical nuclei and indistinct cell walls (Fig. 4).

(*b*) There are usually few eggs present in any one acinus of the gonad, but these are firmly attached to the wall. They move into the lumen only when fertilization is about to take place (Fig. 2).

(*c*) The male germ cells are present in all stages of maturation, and spermatozoa are neatly arranged with their heads attached to a nurse cell and their tails oriented in parallel rows towards the lumen of the gonad (Fig. 3).

Old animals quite often show deviations from this orderly arrangement of the cells of the ovotestis, as well as anomalies in the maturation of the male germ cells, but these are few in number in comparison with those occurring in treated animals.

Methods

The animals were collected a few miles from Halifax and were put into glass dishes containing damp vermiculite. They were kept for a week before being used, in a basement laboratory with subdued light (15–50 m-c) for normal day lengths. Control and experimental animals were fed on fresh lettuce daily.

The method of illuminating the animals is illustrated in Fig. 1, which shows the container in section. An orange crate was placed in an upright position, after a 6-in. square had been cut out of the center partition. The hole was covered by a diaphragm made of black paper, in which holes had been punched; this insured even illumination over the required area. A water cell, consisting of a pyrex dish containing 2–5 cm of distilled water, was placed over this

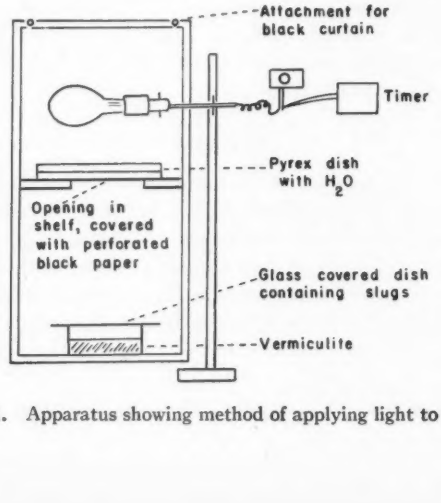

FIG. 1. Apparatus showing method of applying light to slugs.

central hole. If light filters were used, they were placed between the water cell and the hole.

The lights were clamped to a ring stand. The sides of the crate were lined with asbestos and during the experiment the front of the crate was covered by a black curtain. A fan was used to prevent any rise in temperature when the light intensity was above 1000 m-c.

The lights used were General Electric Daylight bulbs of 60 and 100 watts. These bulbs are designed to give an emission spectrum of the solar radiation in New York at 12 midday in midsummer. The intensity of the light was measured as meter-candles with a Weston photographic analyzer, while the daily light period was controlled by a Paragon Electric Timer.

Experimental

Forty animals were used in each experiment with the same number kept as controls. After an experiment the ovotestis was dissected out from each animal and divided; half of the gonad was dropped into fixative, to be sectioned later, while the remainder was used for making a preparation of living cells, which was examined under the oil immersion lens of a phase contrast microscope and if necessary, photographed. The isolated living cells were also compared with those appearing in sectioned and stained material, in order to get a clear idea of the gonad as a whole.

Preliminary experiments showed that 1000 m-c was adequate to bring about visible changes in the germinal epithelium within a reasonably short time. In the first set of experiments, summarized in Table I, light intensities ranging from 1500 m-c down to 50 m-c for 10 hours a day were used. The experiments were continued for 5 weeks. This range of intensities produces the same general type of change in the ovotestis: a rapid rate of maturation of the male germ cells, without marked effects on either cytokinesis or cell constituents. So rapid is this maturation of the spermatogonia that the gonad contained mostly late stages in such large numbers that there were not enough available nurse cells to which the sperm heads could become attached, with the result that the sperms were scattered untidily in the gonad. The germinal epithelium is evidently sensitive to these changed conditions, as it was to the low temperatures used in the previous work cited (1). The original thin layer of cells (Fig. 4) became much wider and the nucleus moved to the side of the cell adjacent to the lumen of the ovotestis (Fig. 5). The thickening was later followed by an increased production of nurse cells, which may account for the young eggs being released prematurely into the lumen, with a breakdown of their cytoplasm. In a few cases, naked nuclei were seen lying in the gonad.

The results of the second group of experiments are summarized in Table I, as II, III, IV, and V. In these, 1000 m-c of light were used for varying lengths of exposure. The results differ from the previous one, in that constant light during the whole day affected cytokinesis. There appeared large numbers of binucleate spermatocytes (Fig. 6) and multinucleate spermatids (Figs. 7, 8, and 9) in a generally untidy gonad. The same intensity used for 5 hours

TABLE I

The effect of light on the ovotestis in slugs

Expt. No.	Light intensity and length of exposure	Duration of experiment	Effect on germinal epithelium	Effect on ♀ germ cells	Effect on ♂ germ cells
I	10 hours daily (*a*) 1500 m-c	1–2 weeks	Width of cell increased; no change in shape	Oogonia with vacuolated cytoplasm and crevulated edges	
		3–5 weeks	Greatly thickened and many nurse cells present. In a few follicles, nurse cells outnumbered other types of cells	Cytoplasm disintegrated in some cases, leaving naked nuclei. Oocytes unaffected	Most of the cells are late spermatids and mature spermatozoa, haphazardly arranged in the gonad
	(*b*) 1000 m-c	1–5 weeks	Same changes as in (*a*), but not quite as extensive, since some normal follicles were still present	As above in (*a*)	As above in (*a*), but including some normally arranged cells
	(*c*) 500 m-c (*d*) 100 m-c (*e*) 50 m-c	1–5 weeks	Same as in (*a*) and (*b*)	As above in (*a*)	As above in (*a*) and (*b*)
II	24 hours daily, 1000 m-c	3–7 days	Epithelium is thickened	Vacuolated cytoplasm in oogonia. Outline of cell is irregular	Large numbers of binucleate spermatocytes and multinucleate spermatids
		3–4 weeks	Thick layer of nurse cells	Same as at end of 3 weeks	
III	18 hours	Same results as with 24 hours			
IV	5 hours, 1000 m-c	Up to 5 weeks	Normal	Normal	Normal
V	2 hours	Up to 5 weeks	Normal	Normal	Normal
VI	24 hours darkness, except while cleaning and airing in subdued light	2 weeks	No change	Cytoplasm vacuolated in oogonia; a single one was lying free in the gonad	No change
		5 weeks	A thick layer of nurse cells, sometimes in a double layer		Many late stages of maturation, untidily arranged. Some follicles almost completely filled with nurse cells and a few scattered spermatocytes
VII	19 hours dark interrupted by two periods of light of total length 5 hours	5 weeks	Ovotestis quite normal	Normal	Normal
VIII	1800 m-c 2000 m-c 3000 m-c 5500 m-c	3 days	Thickening of cells and formation of nurse cells		Cytokinesis and cell constituents markedly affected in primary spermatocytes, which mature directly, giving multinucleate spermatids, yielding nonmotile sperms
		1–2 weeks	Nurse cells in large numbers	Oogonia with disintegrating cytoplasm and detached from the wall of the follicle	

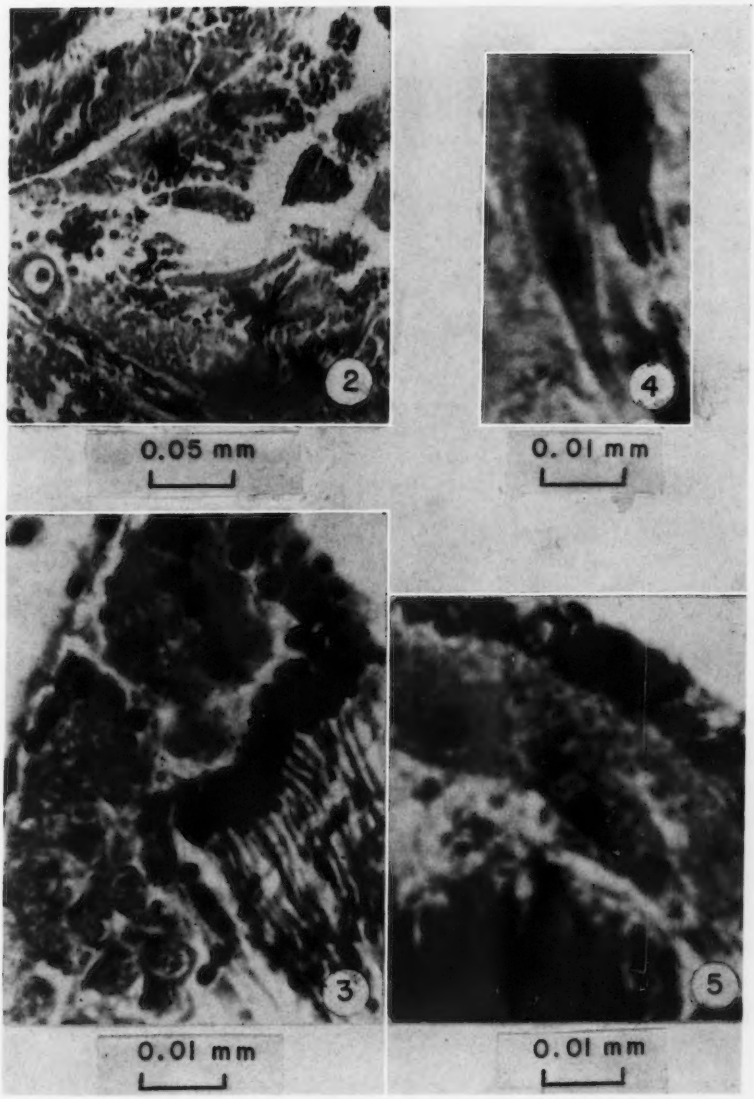

FIG. 2. Section of normal follicle, showing two oocytes and many spermatozoa. Iron haematoxylin.

FIG. 3. The same section, at higher magnification, illustrating the orderly arrangement of sperms.

FIG. 4. Germinal epithelial cell lying inside pigmented layer of the gonad. Two nucleoli and several chromatin blocks are visible. Iron haematoxylin.

FIG. 5. Section of germinal epithelium from an animal exposed to 1000 m-c. The cytoplasm has increased in width. Haematoxylin.

PLATE II

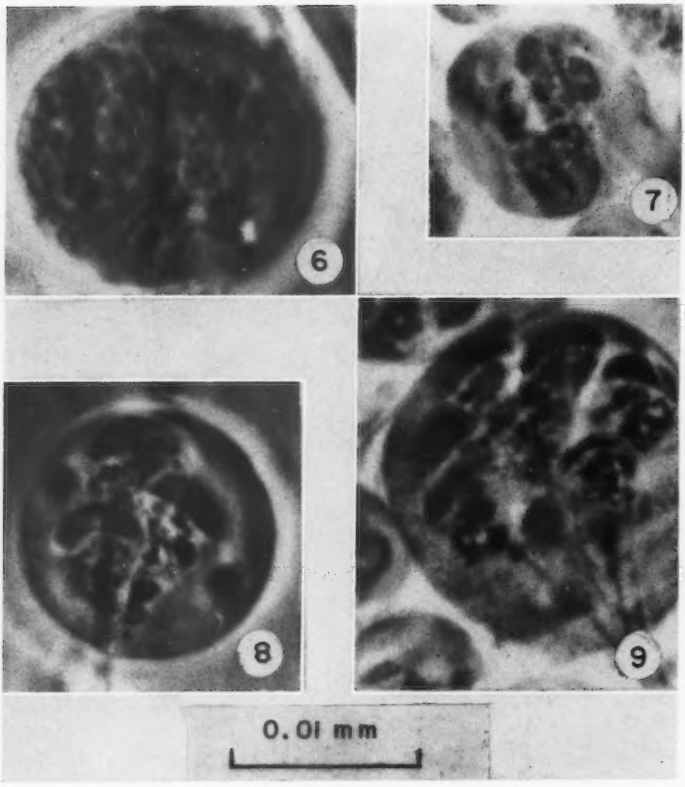

FIG. 6. Binucleate spermatocyte. Live preparation of animal exposed to high light intensity. Phase contrast.
FIG. 7. Binucleate spermatid, in which the nuclei are oriented in opposite directions. Live preparation. Phase contrast.
FIG. 8. Trinucleate spermatid. Live preparation. Phase contrast.
FIG. 9. Spermatid containing 10 nuclei and 10 axial filaments, of which 7 are in focus. Live preparation. Phase contrast.

or 2 hours daily produced no changes at all. Perhaps one might have assumed that a typically nocturnal animal like the slug would produce quite normal cell organization with no light at all; but experiment VI, in which the animals were kept in the dark, except during a very brief exposure to 50 m-c while being cleaned and fed, showed that slow changes occurred, first in the oogonia and later in the ovotestis as a whole. The most striking changes were the production of a double layer of nurse cells and the reduction in the number of early stages of maturation of the male germ cells.

A comparison between animals subjected to light for 24 hours and to no light, or 24 hours in darkness, shows that the effects are different. The former upsets all the cells in the gonad, whereas the lack of light for 24 hours has a marked effect on the germinal epithelium, which differentiates one kind of cell, rather than the three types usually present, suggesting that the mechanism which is normally responsible for inhibiting nurse cell differentiation is no longer able to function.

Since the ovotestis showed marked changes when the animals were exposed for 18 and 24 hours at 1000 m-c, it was to be expected that the rate of change would be accelerated if higher light intensities and shorter exposures were used. Experiment VIII shows that intensities from 1800 m-c to 5500 m-c could produce a thickening of the germinal epithelium and the development of nurse cells in large numbers within 3 days and the other changes described in experiments II and III were evident within a week.

Throughout these experiments the slugs survived just as well as the controls, the death rate being about the same in both groups.

When the animals were returned to their usual environment after being exposed to 1800 m-c for 24 hours a day, for 2 weeks, a few animals were examined. Live preparations showed masses of spermatids, almost all of which were multinucleate, derived from spermatocytes which were mostly binucleate. Sections of some of these animals showed a somewhat untidy appearance. At the end of 6 weeks the few remaining animals were examined and they were quite normal and indistinguishable from the controls.

Summary and Conclusions

1. The cells of the ovotestis of the slug exposed to light intensities from 1500 m-c to 50 m-c for 10 hours daily showed an accelerated rate of the maturation of the male germ cells, accompanied by an untidy arrangement of the contents of the ovotestis. There was no interference with cell division, and spermiogenesis proceeded normally.

2. If the duration of the exposure to 1000 m-c is increased to 24 or 18 hours daily, there is interference with cytokinesis and there is a marked tendency for meiosis to proceed without cell division. This results in the production of many spermatids having two, three, four, five, or six nuclei surrounded by a common cytoplasmic envelope.

3. The germinal epithelium, from which the spermatogonia, oogonia, and the nurse cells are derived, is the first tissue to be affected by exposure to cold

or light. The formation of unusually large numbers of nurse cells under experimental treatment suggests that the underlying control which normally limits their production has been interfered with and this in turn may be responsible for the premature release of the oocytes from the acinar wall.

Acknowledgments

This work was assisted by a summer grant from the National Research Council, to whom we are grateful.

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THE FIRST FILLING OF THE SWIM BLADDER IN SALMONOIDS[1]

J. S. TAIT

Abstract

Salmo trutta, *S. gairdneri*, *Cristivomer namaycush*, and *Coregonus clupeaformis* failed to fill their swim bladders while being reared without access to an air surface for 84, 50, 22, and 56 weeks after hatching, respectively. When given access to an air surface they filled them. It is concluded that, like other physostomes, they must swallow air for the initial filling, but even if filling is delayed their pneumatic ducts remain open. In control trout, filling occurred at the beginning of feeding, and in whitefish controls, 2 to 3 months after hatching (at 20–22 mm length). Lake trout fry with unfilled swim bladders swam up vertical distances ranging up to 900 ft without marked fatigue, indicating that fish hatched in deep water can swim to the surface with relative ease to fill their swim bladders.

Introduction

One aspect of the problem of gas deposition in the swim bladder of fish is the initial filling of the swim bladder of young fish. In newly hatched fish the swim bladder, although well developed at this stage in some species, does not contain any gas. Generally, its filling is coincident with the disappearance of the yolk sac and the beginning of a free-swimming, food-seeking existence.

The present paper is concerned with the first filling of the swim bladder in the brown trout (*Salmo trutta*), rainbow trout (*S. gairdneri*), lake trout (*Cristivomer namaycush*), and whitefish (*Coregonus clupeaformis*). Experiments were carried out to answer four questions. Firstly, are the last three species dependent on the swallowing of air for the initial filling of the swim bladder as are other physostomes investigated to date and as von Ledebur (9) showed the brown trout to be? Secondly, if this is so, can these four species survive when prevented from filling their swim bladders? Von Ledebur's fish that were reared without access to the surface died within a few days to 2 weeks after the normal time of swim bladder filling. The deaths of his brown trout were precipitated by a mechanical failure and were not caused directly by failure to fill their swim bladders as Plattner (13) implied when reviewing von Ledebur's work. Thirdly, if filling at the normal time can be prevented, do these species eventually lose the ability to fill their swim bladders as some physoclists do? Von Ledebur found that if *Gasterosteus aculeatus* and *Lebistes reticulatus* were prevented from reaching the surface for a few days their pneumatic ducts closed and then their swim bladders never filled. Jacobs (6) reported the same for the sea horse (*Hippocampus*). These are physoclists, but it is possible that through disuse the pneumatic duct in physostomes might also close. The final question concerns the ability of young fish to swim

[1]Manuscript received October 9, 1959.
Contribution from the Laboratory for Experimental Limnology, which is operated jointly by the Ontario Department of Lands and Forests and the Department of Zoology, University of Toronto, Toronto, Ontario.

vertically upward without the buoyancy afforded by a filled swim bladder. Fish hatched from demersal eggs in deep water may not be able to fill their swim bladders other than by a long ascent to the surface. This may be an exhausting and hazardous trip for these fish at a critical time in their lives. Do they make the trip with considerable difficulty or with relative ease?

The first three points are of extreme interest in the case of salmonoids hatched in shallow water and in small lakes if the time of spawning and the rate of development are such that the stage at which swim bladder filling takes place is reached before the ice cover leaves the lakes. All four are of interest in the case of salmonoids that spawn in deep water. The problems of ice cover and deep water do not apply to brown trout and rainbow trout, which spawn in streams (Greeley (3) and Hobbs (5)), the latter in spring. They were used in the present experiments because they were readily available representatives of the salmonoids. Both ice cover and deep water could be problems for lake trout and whitefish. Pertinent data on time and depth of spawning for these and some related species are presented in Table I. The single lake trout fingerling captured by Royce (14) on April 2, 1940, at a depth of 130 ft in Seneca Lake might have encountered ice if it had swum to the surface to fill its swim bladder. Royce estimated that the time of hatching was late January.

TABLE I

Time and depth of spawning of some salmonoids

Species	Lake	Time of spawning	Depth of spawning (ft)	Author
Coregonus clupeaformis	Lake Huron	Oct. 25–Nov.	4–48	Koelz (7)
Coregonus clupeaformis	Lake Ontario	Nov.–early Dec.	0–90	Koelz (7)
Coregonus lavaretus (pelagicus)	Lake Constance		500–800	Kriegsmann (8)
Cristivomer namaycush	Lake Louisa	Oct. 24–Nov. 7	1–4	Martin (11)
Cristivomer namaycush	Lake Nipigon	Oct. 1–Oct. 30		Dymond (1)
Cristivomer namaycush "deep water trout"	Lake Nipigon	Oct. 20–Nov. 10	120–180	Dymond (1)
Cristivomer namaycush	Seneca Lake	Sept. 23–Nov. 3	100–200	Royce (14)
Cristivomer siscowet	Lake Superior		Deep water	Milner (10)
Cristivomer siscowet	Lake Superior	June–Nov.	300–480	Eschmeyer (2)
Leucichthys hoyi	Lake Michigan	March	170	Koelz (7)
Leucichthys kiyi	Lake Michigan	Oct.	420	Koelz (7)
Leucichthys nigripinnis	Lake Superior	Sept.	360–600	Koelz (7)

Materials and Methods

Eggs of lake trout, rainbow trout, and whitefish were supplied by Ontario Provincial Hatcheries. Additional whitefish eggs were obtained from whitefish netted in Lake Ontario. Brown trout eggs came from stock maintained at the laboratory. Trout eggs were incubated in laboratory troughs. Whitefish eggs were incubated first in hatching jars and later were transferred to troughs. Just before hatching, the eggs that were to provide fry for the experiments were transferred to special rearing containers. For controls some trout fry of the three species were reared in troughs and whitefish fry were reared in an aquarium.

Three types of container which prevented access to the surface were used to rear the young fish. The first consisted of a battery jar closed about 3 cm below the top with a screen of cotton gauze or aluminum wire. Water entered at the bottom and overflowed at the top. Two of these were used in experiments on whitefish and lake trout in 1955. The second type was a rectangular cage of aluminum screening immersed in a large tank of water. Six of these were used for rearing rainbow trout in 1955. The third type was a 3½-gal bottle with an aluminum screen held in the neck by two rubber rings. Water entered at the bottom through a glass tube inserted through the screen and overflowed at the top. These were used for experiments begun in 1956. The bottles were much superior to the earlier containers. They facilitated observation of the fish, and the advantage of good circulation coupled with a small screened outlet (4 cm in diameter) made them partly self-cleaning.

To prevent the formation of gas bubbles in the containers the tensions of dissolved gases in the water supply were reduced slightly below saturation levels by aerating water at 9°–14° C in a gas exchange column and then cooling it to 4°–9° C in countercurrent cooling tubes.

The trout were fed ground beef liver, liver mixed with a commercial turkey feed, and sometimes enchytraeid worms. Whitefish were fed *Artemia* at first and later enchytraeids.

The normal time of swim bladder filling was determined by observation of the control fish. The presence of a filled swim bladder is usually apparent from the buoyant swimming behavior of the fish. The time of filling was more definitely established by examining fish from time to time either by dissection or by pipetting up live fish in a glass tube and placing them in front of a bright light. In small fish when a swim bladder is filled it is readily visible as a silvery blue sac dorsal to the gut. The pipette method was used for removing and examining fish confined under screens. In this way a fish could be taken from the tank, examined, and returned without being exposed to an air surface. Dead fish were dissected unless they were partly decomposed. When a large number of fish died at one time approximately half of them were examined. Occasionally, fish with unfilled swim bladders were released in a tank with an open surface to determine whether they were still capable of filling their swim bladders. When fish became too large to examine with a light they were X-rayed before and a few days after being released in a tank with an open surface. Dental X-ray facilities were very kindly made available by Dr. M. N. Rockman. The fish were transported to the X-ray machine in erlenmeyer flasks closed with perforated stoppers and submerged in a water bath. They were anaesthetized with tricaine methanesulphonate for transfer from the rearing bottles to the flasks and again from the flasks to X-ray plates. The plates were DF45 dental occlusal super speed film. They were exposed for ¾ sec to 70 kv, 10 ma.

The methods for the vertical swimming experiments are described with the results of these experiments.

TABLE II

Observations on the time of first filling of the swim bladder in control fish and on the failure of fish to fill their swim bladders when reared without access to an air surface

Species	Controls		Reared under screens		
	Mean temp. (°C)	Time of swim bladder filling (weeks after hatching)	Mean temp. (°C)	Swim bladders unfilled (weeks after hatching)	No. examined
Cristivomer namaycush 1955	10	4–5	10	16	12*
Cristivomer namaycush 1956	2	9	5.5	22	18
Salmo gairdneri 1955	9	3½	9	6½	241*
Salmo gairdneri 1956			6	50	39
Salmo trutta 1956	3	17	3	84	133
Coregonus clupeaformis 1956	4.5	15 (20–22 mm in length)	6.5	56	37

*Some of these had gas in their swim bladders. Presence of gas explained by accidental availability of an air surface at various times.

Observations and Results

Control Fish

The times of swim bladder filling in the control fish are shown in Table II. Lake trout, rainbow trout, and brown trout remained on the bottom of the troughs while they had a prominent yolk sac. By means of vigorous tail movements they sometimes moved about rather awkwardly and occasionally darted up into mid-water, then fell back again. When the yolk sac was almost absorbed, at first one or two fish and eventually all were observed swimming in mid-water or near the surface, suspended horizontally, requiring little swimming effort except that needed in moving from place to place or in maintaining their position in a current. Their swim bladders quite obviously were filled. Interest in feeding began at this time.

The whitefish fry behaved quite differently. This may be associated partly with their small yolk sac on hatching. As soon as the fry freed themselves from their egg capsules, they swam up to the surface and swam along with their heads just beneath the surface, occasionally breaking through. At first this was regarded erroneously as an attempt to fill their swim bladders. Even though their swim bladders were not filled until much later the fry swam about in mid-water apparently with considerable effort. Very rarely were they seen on the bottom. The yolk supply lasted for a few days or a week and then they began feeding on *Artemia*. Ingested food could be seen easily in the gut. Some of the control fish hatched prematurely because of mechanical agitation in the hatching jars. They had abnormally large yolk sacs and did not begin feeding for 4 weeks. Before the swim bladders were filled gas bubbles were observed in the gut in 50% of the fish examined. When the swim bladders were filled, swimming movements became much more leisurely.

Fish Reared Under Screens

The results shown in Table II answer the first two questions for which the experiments were designed. These four species are dependent on swallowing

air for the initial filling of their swim bladders, but they can survive even though their swim bladders remain gas-free. Concerning the third question, there is no doubt that the pneumatic ducts remained open in the fish reared away from the surface. The ducts were still patent in the 1956 lake trout at the 22nd week when the experiment was ended by the accidental occurrence of an air surface, and in the other three species when the experiments were terminated. The X-rays of these three species taken at the end of the experiment showed that the swim bladders were empty. X-rays of the same brown trout and rainbow trout taken several days after they were released in open tanks showed that the swim bladders were then filled (Fig. 1). Only two whitefish were X-rayed a second time and this was done 1 hour after their release in an open tank. One had begun to fill its swim bladder. Some of the X-rayed whitefish were dissected a few days after their release. All of these were found to have filled, normal-looking swim bladders except one individual that had a small J-shaped bladder about one half the normal length and containing one small bubble of gas. Four brown trout were left in the rearing bottle after the others were X-rayed at 63 weeks. They died accidentally at 84 weeks. Their swim bladders were well developed and they were easily inflated by means of a pipette inserted in the esophagus.

There were marked differences in behavior among the four species reared in bottles. The whitefish swam continuously assuming a vertical position with head up. Although they were obviously heavy and sank when they momentarily ceased swimming, they rarely were seen resting on the bottom. The lake trout and the rainbow trout alternated between swimming upward and resting on the bottom. The lake trout tended to swim around the periphery of the bottle at an angle of 45°, occasionally swimming vertically just under the screens. The rainbow trout swam in a vertical position whether at the top of the bottle or in the middle, keeping themselves suspended with rapid thrusts of their tails. The brown trout made no attempt to maintain themselves in the water. They remained on the bottom with pectoral fins outstretched, making occasional darting movements either when frightened or while feeding. All of the brown trout were in excellent condition in contrast with some of the lake trout, rainbow trout, and whitefish which were quite thin.

The behavior of the fish immediately after their release from the screened bottles was extraordinary. They swam rapidly up and down between the bottom and the surface, breaking the surface, and snapping at the air. This excitement brought on by the discovery of an air surface was most marked in the whitefish. They swam vertically at the surface either in circles or back and forth along the full length of the tank with their heads at times thrust out of the water. This continued for several hours and in some individuals for a day or two even after they had partly filled their swim bladders. In most cases released fish of all four species were swimming in a normal manner a few hours or at the most a day after release.

TABLE III

Results of experiments to measure the ability of lake trout fry with unfilled swim bladders to swim vertically upward

Vertical distance (feet)	Time (minutes)	Average temperature (°C)	Average rate (feet/minute)
105	30	12	3.5
111	17	12	6.5
156	35	11	4.5
201	44	13	4.6
498	90	14	5.5
900	129	13	7.0

Vertical Swimming Experiments

The persistent attempts of the fish reared under screens to swim upwards suggested the following simple experiment. A lake trout was permitted by means of a temporary connecting tube to swim up from the rearing bottle into a lucite tube (4.5 ft × 2 in.) completely filled with water and stoppered at both ends. The tube was held vertically with two hands while the fish swam toward the top. When it approached a marker near the top, the tube was rotated vertically through 180°. The fish righted itself and continued swimming upwards. Only the middle section of the tube between two markers 3 feet apart was used as the swimming course. It was found at the beginning that if the fish was allowed to reach the top, which became the bottom on rotation, it would often rest for a time on the bottom. This type of rest could not be permitted. However, the fish sometimes did rest by remaining poised at one level or by stopping swimming completely and falling perhaps 2 or 3 feet. This is a rest from activity that could occur in nature when a young fish is

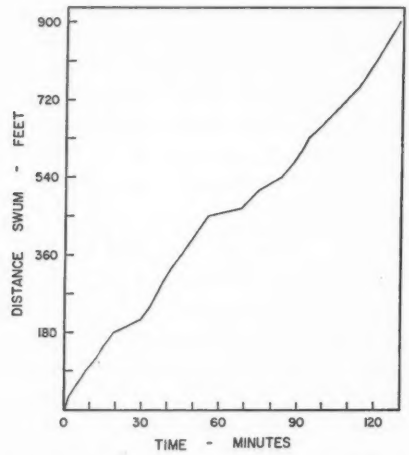

FIG. 2. Graph showing the rate at which a lake trout fry with unfilled swim bladder swam vertically upwards.

PLATE I

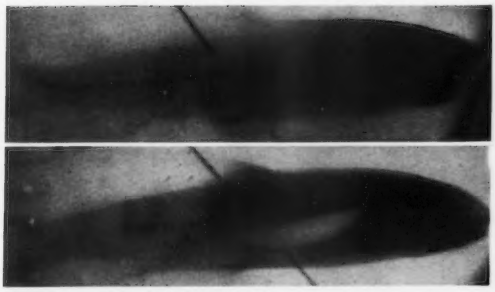

FIG. 1. X-ray photographs of a rainbow trout reared without access to an air surface. (Upper) Swim bladder empty. (Lower) Swim bladder filled after fish released in an open tank.

swimming from the depths to the surface. This procedure was carried out with several lake trout that were about 6 weeks past the normal time of swim bladder filling. The results are shown in Table III. The distances recorded are not maxima for the individual fish. All of the fish were swimming upwards when the runs were terminated except one that was frightened by a bubble and persisted in swimming to the bottom. The bubble had been admitted to the tube to induce the fish to swim up. It had been observed that a fish would often follow but could not overtake a small bubble as it rose through the water. In this case the bubble was accidentally much too large. Some of the fish were swimming at a slower rate at the end while the one that swam 900 feet had about the same swimming rate at the end as at the beginning (Fig. 2).

Discussion

The times of first filling of the swim bladder observed in these four species correspond with the few observations that have been made on other salmonoids. In the Salmonidae the essential point of correspondence is that filling takes place at the stage of development associated with the disappearance of the yolk sac. The actual time measured in days or weeks after hatching depends on the rearing temperature. Von Ledebur's (9) brown trout filled their swim bladders at 3 weeks. The temperature is not given but it must have been much higher than that at which the present brown trout were reared. An average temperature of 3° C is lower than brown trout would normally experience at this stage of development. Hoar (4) reported that *Salmo salar* filled its swim bladder 5 to 8 weeks after hatching. The temperature increased from 0.4° C in the week before hatching to 19° C 5 weeks after hatching. The observations on *Coregonus clupeaformis* coincide with those of Kriegsmann (8) on *Coregonus lavaretus* of Lake Constance. The fry of one ecological type *pelagicus* referred to as "Blaufelchen" fill their swim bladders when they are 22–25 mm long and those of another type *nanus* referred to as "Gangfisch" when 21–27 mm long. The time in both cases is 2–3 months after hatching. Whitefish seem to get along very well during the first 2 or 3 months of their free-swimming existence without the aid of a swim bladder. The gas bubbles observed in the gut before the swim bladder was filled undoubtedly help to make the fish more buoyant.

Although many fish died in these experiments, it should not be concluded that fish with a gas-free swim bladder die more readily than those with filled swim bladders. The high mortality can be attributed to the difficulties met in feeding the fish and cleaning the containers. These difficulties were accentuated by overcrowding. Similarly, the deaths of von Ledebur's fish can probably be attributed to the difficulties involved in rearing them in screened containers, immersed beneath the surface. In a later experiment von Ledebur and Wunder (10) with special care kept alive for 10 months two sticklebacks (*Gasterosteus aculeatus*) that had been prevented from filling their swim bladders. These fish were reared in an open aquarium after the time of swim bladder filling had passed and their pneumatic ducts were no longer patent. In the present experiments mortality practically ceased when the number of fish in each bottle was

reduced to about 30 or less. The generally poor condition noted in three species may have been caused by inadequate feeding, but probably it was caused partly by the large amount of energy expended in vigorous swimming to stay suspended in the water without the aid of a swim bladder. This is supported by the fact that the brown trout spent little time swimming and were in good condition.

The indicated problems relating to ice cover and deep-water hatching may now be reconsidered in the light of these experiments. It is immediately apparent that whitefish are not prevented from filling their swim bladders by an ice cover because swim bladder filling does not occur until 2 or 3 months after hatching. In nature, higher temperatures could bring about swim bladder filling earlier, but their occurrence would deny the presence of an ice cover. On the other hand lake trout hatched from eggs spawned early in the fall likely are ready to fill their swim bladders before the ice leaves the lakes. If the ice cover does cause a delay, the effects probably are not serious since fry seem to be able to swim for long periods without the help of swim bladders, and the ability to fill their swim bladders is not lost. The swimming experiments strongly indicate that fish hatched in the depths of fresh-water lakes can swim to the surface with relative ease. If the siscowets spawn even in the deepest part of Lake Superior, which is approximately 1300 ft (Van Oosten (15)), the fry could reach the surface in about 3 hours, swimming at the rate observed here.

Acknowledgments

It is a pleasure to thank Professor F. E. J. Fry, who suggested this investigation and supported it with characteristic enthusiasm. I gratefully acknowledge the financial assistance of the National Research Council and the assistance of Ontario Provincial Fish Hatcheries in supplying fish eggs. X-ray facilities and advice in their use were very kindly provided by the late Dr. M. N. Rockman of the Faculty of Dentistry, University of Toronto.

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NUCLEAR BEHAVIOR OF BLOOD CELLS[1]

VIBEKE E. ENGELBERT

Abstract

This paper reports the continued investigations into nuclear behaviors and activities of blood cells.

Further proof of the extreme changes in shape of the almost naked nuclei of blast cells and evidence of the motility of such nuclei are presented. Further evidence for the release of intranuclear vesicles which become red blood cells is given. The cyclic nature of the life history of lymphocytes, which was first postulated in 1956 (Engelbert (5)), is again emphasized, also the extreme changes in morphology of the whole nucleus as well as the basic nuclear units which take place during the life history. The nucleus should be regarded as a federated composite structure—a federate autonomy—made up of smaller structures, the basic nuclear units, that each have a certain autonomous potential, which comes into play when they leave the composite federation. The nuclear federation can become very large forming polypoid nuclei. Naked nuclei can form "cytoplasm" by contracting and thus squeezing nucleoplasm out into a rim around the nucleus.

Introduction

Earlier papers (5, 6) by Engelbert demonstrated that blood cell development depends on the behavior of lymphocyte nuclei and the life history followed by them. The life history of the lymphocyte can be cyclic. It was shown that fusions of lymphocyte nuclei gave rise to large composite mother nuclei. These large nuclei were called myeloblast nuclei because of their close similarity to nuclei of myeloblasts in the literature. However, the term blast cell will be used here instead of myeloblast. The large blast cell nuclei were shown each to give rise not only to one type of blood cells, but to several by a process not described before. This process allows nuclear structures produced by the blast cell to become free and form new cells. In this way very small new lymphocytes are released as small chromatin masses (basic nuclear units) surrounded by a nuclear membrane but at first showing no cytoplasm. Young neutrophiles can be released as masses of granular nucleoplasm which contain small chromatin bodies that will become the nuclei. Intranuclear vesicles with a semifluid content and a firm outer membrane are also released by this very productive blast cell nucleus. Many of the released vesicles become red blood cells. In live preparations one can observe that the activity of the blast cell nucleus is very dynamic. The nucleus extends and contracts, while its chromatin divides at least three times to form the bodies which become free basic nuclear units (5). The nucleus can also move back and forth with a spiralling movement and at this time it releases intranuclear vesicles. Blast cell nuclei come in many different sizes. The very large ones produce several kinds of new blood cells. The smaller blast cell nuclei may only give rise to one or two kinds of new blood cells. It would be valuable if we determined the sizes of the various blast cell nuclei and gave them a corresponding number and so kept track of their sizes instead of using many different names, which only leads to

[1]Manuscript received August 22, 1959.
Contribution from Department of Zoology, University of Toronto, Toronto, Ontario.

confusion and misunderstanding. Eosinophils and basophils are also formed from components of blast cell nuclei. Lymphocytes can transform directly into plasma cells under the influence of foreign protein (10). The young lymphocytes, as mentioned above, ordinarily fuse to form the larger blast cells, but under the influence of foreign protein, lymphocytes can release their basic nuclear units and thus start a new developmental cycle directly. It is as if the lymphocyte is placed on a chemical pivot, which can be turned in various cytogenic directions of development through the influence of hormones, foreign proteins, and other substances present in blood and tissue fluid. In this way this highly potential cell can provide for the production of the type of blood cells which the body needs under various circumstances.

When one observes the dynamic behavior of the large blast cell nuclei in live preparations, namely their movements and release of vesicles, etc., one is constantly reminded of the behavior of certain protozoa. We have long ago through Metchnikoff's work accepted certain protozoan behaviors as characteristics of certain blood cells, namely amoeboid movement and phagocytosis. It would not seem unreasonable to expect that other protozoan characteristics were possessed by vertebrate blood cells.

The above process of development of new cells from the blast cell means that most of the substance of the mother cell nucleus is spent (usually a part of the nuclear chromatin remains). The phenomenon whereby parts of the mother cell nucleus can be released has by most authors been regarded as a "breakdown process", a process of cell destruction and not a process of consequent cell regeneration, such as described here.

In order to understand the long and intricate process of blood cell cytogenesis, we must unravel the stages of the lymphocytic and blast cell history bit by bit. One of the most confusing aspects of the blood cell history is created by the many variations in nuclear behavior which do not fit classical descriptions of blood cells. The writer believes that only by following the nuclear behaviors, however unorthodox they are, can we piece together the puzzle of blood cell development. Observations on nuclear behaviors both in lymphatic and in certain glandular tissue are reported in the following pages.

Materials and Methods*

The spleens, lymph nodes, and livers of rats and rabbits were used for the present work. The imprint method was used throughout.

Observations

The nuclei which are described in the following pages appear in all but a few cases devoid of cytoplasm. We recognize that the lymphocyte has very little cytoplasm and sometimes in ordinary blood smears one cannot see even a narrow rim of cytoplasm around the lymphocytic nuclei. It was pointed out previously by the writer (5, 6) that many of the nuclear stages in the life history of blood cells were characteristically nuclei without surrounding

*For further details see earlier papers (Engelbert (5, 6)).

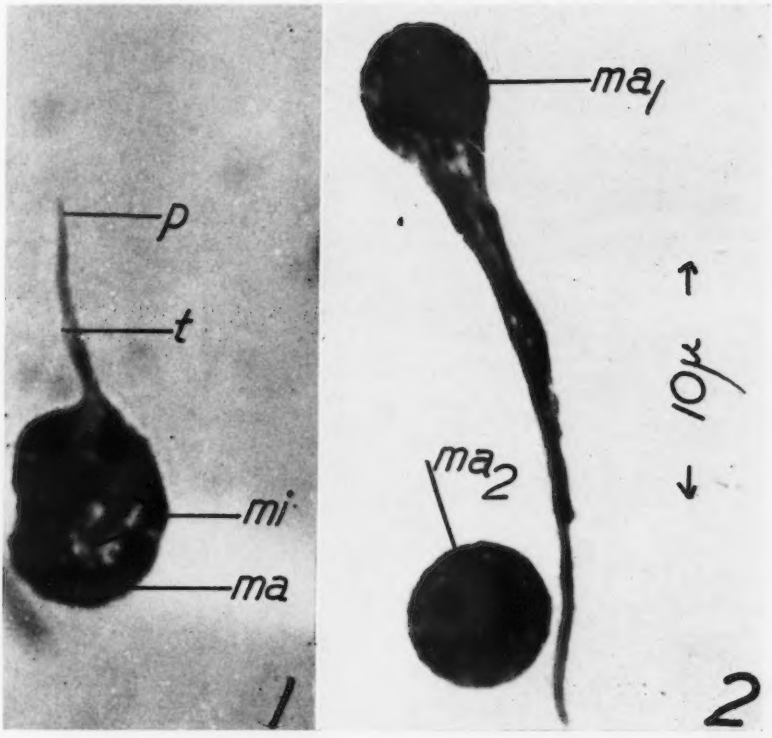
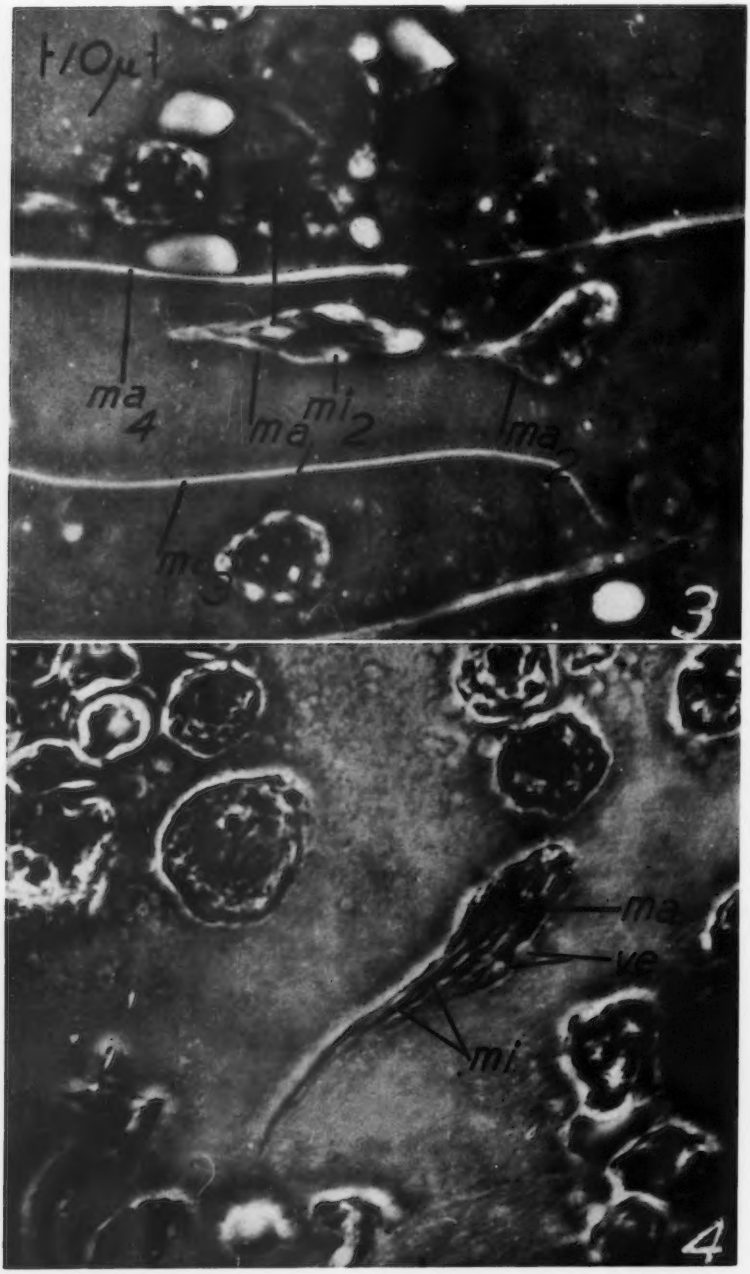


FIG. 1. Blast cell nucleus (*ma*) showing extension, which ends in a point (*p*) and is twisted (*t*). *Mi* is a mass of chromatin which represents a basic nuclear unit. Rat liver. Ac.A. Feulg.

FIG. 2. Elongated (ma_1) and rounded (ma_2) blast cell nucleus. Rat liver. Iv. M.G. + G.

PHOTOMICROGRAPHS: Leitz, ortholux, objective 90×, ocular 10× used for Figs. 1, 2, 7, 13, and 14. Reichert, Zetopan, objective 45×, ocular 10× used for rest of figures. Figs. 3, 4, 5, 6, 9, and 12 photographed with phase contrast.

Ac.A.—acetic alcohol 1:3.
Meth.—methanol.
Iv.—Iodine vapor.
Z.A.—Zenker's fixative with acetic acid.
M.G. + G.—May-Gründwald plus Giemsa stain.
Feulg.—Feulgen nucleal stain.



Engelbert—Can. J. Zool.

cytoplasm. If we look for blood cells of the classical types, we look for cells with cytoplasm which possess various degrees of basophilia. In this way we reject all cells (except the lymphocyte) which appear to be almost naked nuclei. This very selection of nuclei surrounded by cytoplasm means that over the years we have selected certain stages in the blood cell life history because it suited our conception of a normal cell. Thus, we rejected all the stages which showed nuclei devoid of cytoplasm and which had odd nuclear shapes and types and did not fit our conception of a proper nucleus. The writer believes this very selection of cells and our rejection of all the "odd looking" cells has prevented us from really understanding the cytogenesis of blood cells. We have in fact only selected a few stages of their life history and consequently rejected the majority of stages, which would have told us the true developmental history.

The writer pointed out previously (5) that blood cell nuclei which appear almost spherical in shape, which are deeply stained, and which have a rim of cytoplasm around them are in a contracted state (Fig. 11, *nuc*).

In live preparations (5) one can see the nucleoplasm being squeezed out from the nucleus quickly during its contraction and this displaced nucleoplasm becomes the cytoplasm. This is a new concept but one which cannot be denied by anyone who has observed the strong contractions of nuclei cultured in vitro and the emerging of the semifluid substance from the nucleoplasm. It often appears that the outermost layer of nuclear membrane remains more or less in the original position and does not follow the contraction. This outer membrane then holds the released nucleoplasm in place as a rim around the contracted nucleus. Several delicate strands may connect the contracted nucleus and the outer membrane (Fig. 11).

A gradual and slow contraction of the nuclei of lymphocytes or blast cells can also be observed in cultures in vitro. The end result of such contraction can be the creation of typical plasma cells (10).

The nuclear extension of blast cells that will be described here always begins with one portion of the nucleus being drawn out as if it were a long narrow "neck" (Fig. 1, *ma*, *p*; Fig. 3, ma_1, ma_2). The portion of the nucleus behind the "neck" usually remains more or less rounded at least for a time (Fig. 2, ma_1; Fig. 3, ma_1, ma_2). The extended portion of the nucleus is often drawn out to so fine and needle-like a point that it is hard to discern except with very careful focussing with the oil immersion objective (Fig. 1, *p*; Fig. 4, *ma*; Fig. 6, *p*; Fig. 10, arrow). The extended portion twists (Fig. 1, *t*) as if spiralling forwards. It should be noted that in preparations fixed in acetic

FIG. 3. Shows blast cell nuclei in various stages of extension from beginning elongation (ma_1, ma_2) to the extreme stretching which form thread-like nuclei (ma_3, ma_4). The nucleus ma_1 clearly shows basic nuclear units of which two (mi_1, mi_2) are labelled. Mi_1 has strands connecting it to other basic nuclear units. Mi_2 is elongating towards the pointed end of the nucleus; it also shows strands which connect it to other basic nuclear units. Rabbit lymph node, 48 hours after injection of 10 ml horse serum. Meth. M.G. + G.

FIG. 4. An elongated blast cell nucleus (*ma*) shows a spear-shaped elongating basic nuclear unit which toward the rounded end of the nucleus is connected to other basic nuclear units. Two vesicles (*ve*) are seen toward one side of the nucleus. One vesicle is being released by the nucleus. From same preparations as Fig. 3, but in deeper focus.

alcohol (1:3) or in Zenker's fixative with acetic acid and followed by the Feulgen stain, the feulgen-positive substance in blast cell nuclei shows the above patterns (Figs. 1, 6, 7) in the same manner as in nuclei fixed with iodine vapor and stained with the May-Gründwald plus Giemsa stain. The spiralling motion which gives the nucleus temporarily a corkscrew shape is clearly shown by the small lymphocyte nucleus (*e–p*), in Fig. 6. The elongation and spiralling motion allows the nuclei to propel themselves through the tissues. It was pointed out earlier (5, Fig. 13) that elongated lymphocytic nuclei would establish contact with each other by twisting the pointed ends around one another like a pair of hooks. Such nuclei can draw the rest of their nuclear mass up towards the point of attachment. Some small lymphocytic nuclei establish contact and later fuse together at their extended portions by first twisting together (Fig. 7, *t*), as if they were individually coiled strands of rope.

The nuclear elongation of blast cells can become so extreme that each nucleus appears as a thin strand (Fig. 3, ma_3, ma_4). In this condition blast cell nuclei can reach a length of 100 μ or more and establish contact with other nuclei that were far away from the original position of the strand-like nucleus. At the time the blast cell nuclei reach this strand-like elongation most of their nucleoplasm is released as vesicles or granules. While considering the tremendous extension of the whole nucleus one has also to follow the fate of the basic nuclear units. In Fig. 3, mi_1 and mi_2 represent two basic nuclear units inside a blast cell nucleus ma_1. Mi_1 shows a solid central mass and fine strands extending out to other units. Mi_2 has an elongated portion along the periphery of the nucleus extending in the direction of the neck-like elongation of the nucleus. In Fig. 4, the arrow-shaped structure (*mi*) resembling a narrow "V" lying on its side is an extended basic nuclear unit which, towards the blunt end of the nucleus, is attached to at least one other basic nuclear unit. It is as if the chromatin representing the units unravels or stretches and several basic nuclear units join up tandem-fashion and form very long feulgen-positive strands.

Smaller nuclei may represent either a single basic nuclear unit or a few units as the one illustrated in Fig. 6 (*e–p*) and the small nuclei (*mi*) shown in Fig. 7.

The behavior of elongation, twisting or fusing together, seem to be of the same patterns whether the nuclei are from small lymphocytes or large blast cells.

The ability of elongated lymphocytic nuclei to move about in tissues is great. In imprints of liver, small masses of liver parenchyma often adhere to the slides as readily as the free blood cells. In such preparations (Fig. 10) one can find many nuclei (probably lymphocytes) in various stages of elongation moving into the liver parenchyma. In Fig. 10 two such elongated nuclei (mi_1, mi_2) are seen lying in the cytoplasm of the liver parenchyma. From observations of elongated nuclei in cultures in vitro, one expects that the nuclei which have migrated into the parenchyma can round up and remain in this location as the usual oval or rounded nuclei commonly seen here.

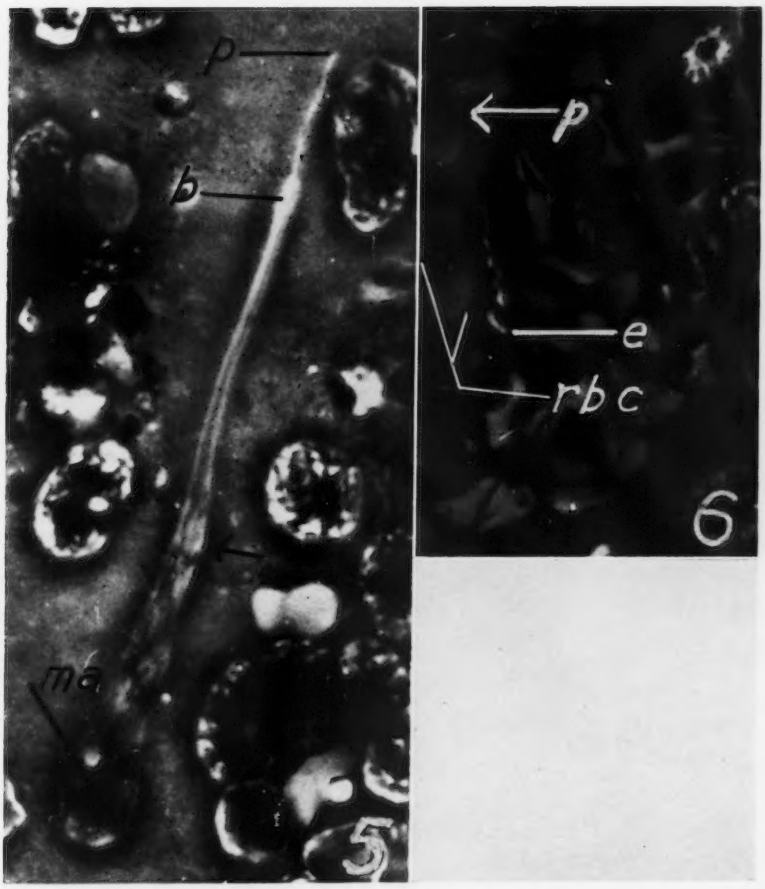


FIG. 5. Elongated blast cell nucleus (*ma*) with thin pointed end (*p*), a bulb-like part (*b*) contains (see arrow) three basic nuclear units, which are extending into long, fine threads. Same preparation as Figs. 3 and 4.

FIG. 6. Arrow (*p*) points to fine needle-like end of the nucleus of a small lymphocyte whose blunt end is shown at (*e*). The nucleus was fixed at a time when it was twisting and moving between red blood cells (*rbc*). Normal rabbit spleen. Z.A. Feulg.

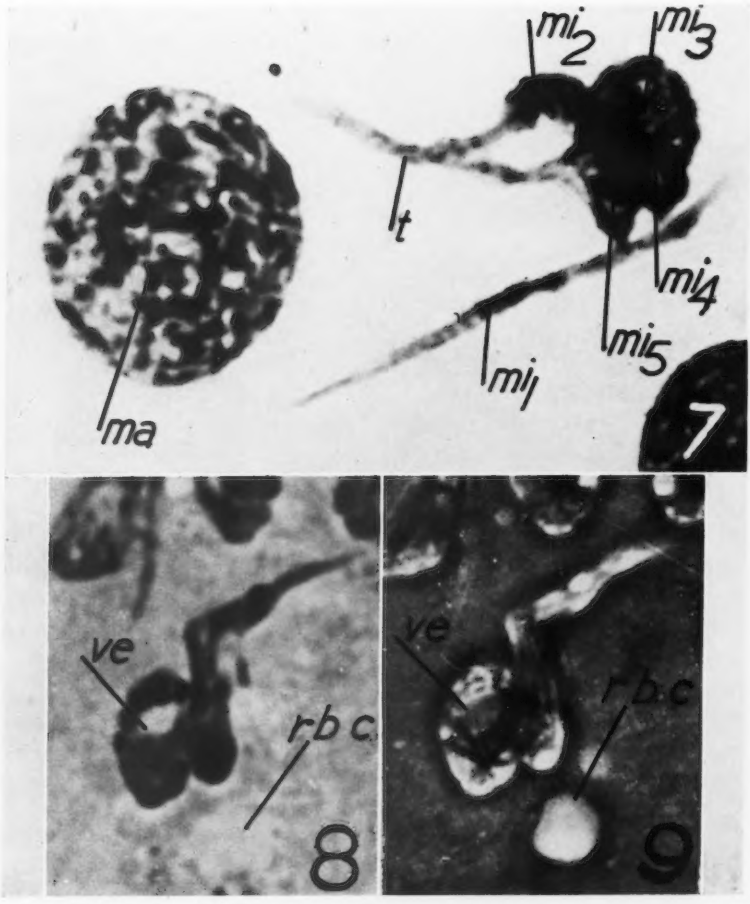


FIG. 7. Blast cell nucleus (*ma*). Small lymphocytic nuclei (mi_1–mi_5). Four of the small nuclei are partially twisted together at (*t*). Mi_1 is still in a fully extended condition. Normal rat liver. Ac.A. Feulg.

FIGS. 8 and 9 from same preparations as Figs. 3 and 4.

Blast cell nucleus with intranuclear vesicle (*ve*). A newly released vesicle (*rbc*) is a young red blood cell.

FIGS. 8 and 9. Photomicrograph with ordinary light (Fig. 8) and with phase contrast of the same field (Fig. 9).

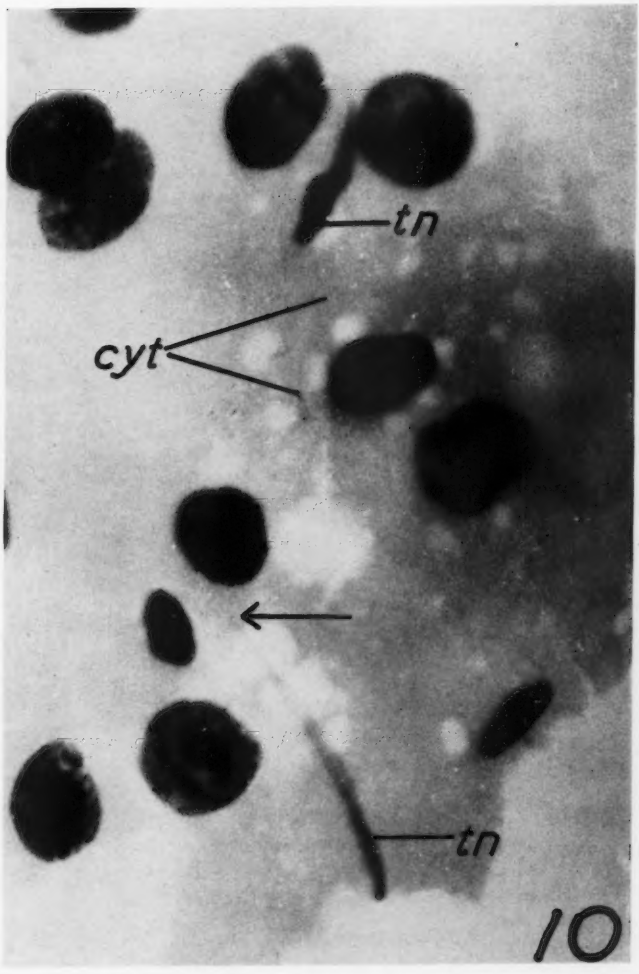
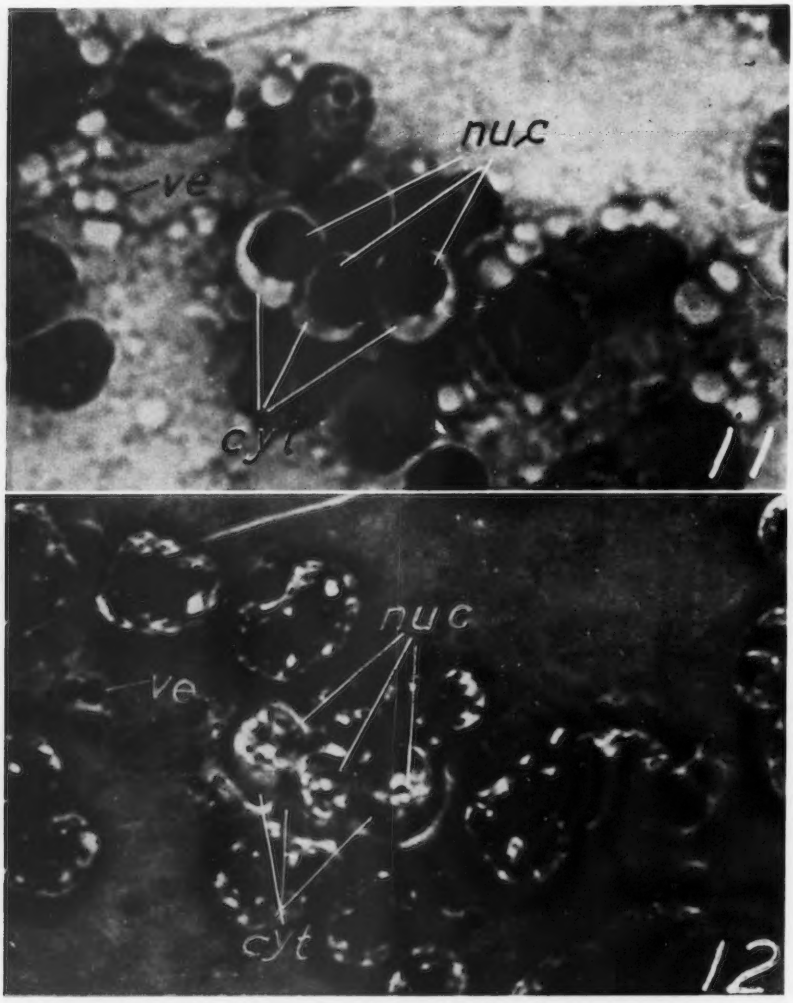
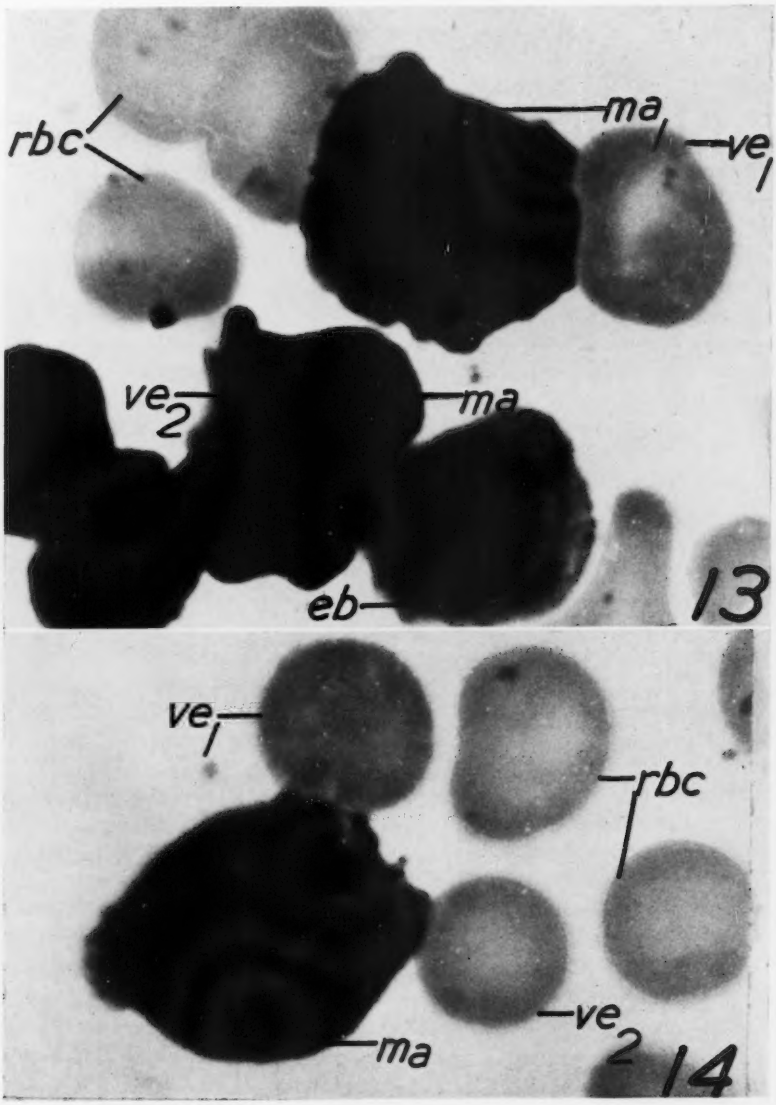


FIG. 10. Cytoplasm (*cyt*) of liver parenchyma. Elongated and twisting lymphocytic nuclei (*tn*) lying in parenchyma. Arrow points to extremely fine point of one elongated nucleus. Normal rat liver. Z.A. Feulg.



FIGS. 11 and 12 show the same field photographed with ordinary light (Fig. 11) and with phase contrast (Fig. 12). Nuclei (*nuc*) of cells similar to classical erythroblasts. Note white rim (*cyt*) is unstained and similar to vesicles (*ve*) in Fig. 11. In Fig. 12 the semifluid consistency of both cell rim (*cyt*) and vesicles (*ve*) is evident.
Lymph node from rabbit injected with 10 ml of horse serum. Meth. M.G. + G.



FIGS. 13 and 14. Blast cell nuclei (*ma*, ma_2) are releasing vesicles (ve_1, ve_2). Red blood cells (*rbc*), classical erythroblasts (*eb*). Liver imprint, normal ♀ rabbit 6 weeks old. Meth. M.G. + G.

The extreme changes in nuclear morphology just described are significant in relation both to the function of blood cells and to the relative phase of blood cell development which such nuclei represent. The nuclei are able to move, to attach themselves to other nuclei, and finally to fuse (after the initial attachment) with other nuclei so that a much larger nuclear element is consequently built up. The larger the nuclei, the greater is the cytogenic potential for releasing many basic nuclear units and producing lymphocytes. Many vesicles which form red blood cells (6), and several granular masses of nucleoplasm containing small nuclei (5) which lead to formation of neutrophils, can also be released from the large nuclei. The production of eosinophils and basophils seems in part (5, 6) to depend on presence of foreign proteins as both cell types become more numerous in spleens from rabbits injected in this laboratory with foreign protein. The origin of these two cell types can arise in part from remnants of blast cell nuclei that earlier have released most of their substance but there is also a possibility that they can arise as "reassociation cells" (6) by combination of vesicles, granules, and nuclear portions released during blast cell activity. The kind of protein which is provided for the blast cell nuclei to work with can no doubt determine what products these nuclei can synthesize and consequently release.

Another important nuclear behavior found in the blood cell series is nuclear contractions. These contractions can take place quickly (5) or slowly (10), but usually some of the nucleoplasm becomes squeezed out of the nucleus during the contraction. The released nucleoplasm is semifluid and sometimes contains very fine granules. At the time of the nuclear contraction the outermost layer of the nuclear membrane may not take part in the contraction. Instead, this outer membrane remains more or less in the position which it held before the contraction, and thus it prevents the nucleoplasm which is squeezed out from escaping. In this way a neat rim of "cytoplasm" is formed around the contracted nucleus. The outer membrane, which now is the cytoplasmic membrane, often has delicate strands connecting it to the contracted nucleus (Fig. 11, *cyt*). Sometimes the rim of squeezed-out nucleoplasm (new cytoplasm) may stain very poorly (Fig. 11) while in other cases it shows a basophilia. The semifluid character of the released nucleoplasm can be seen in Fig. 12, where vesicles (*ve*) and nucleoplasm (*cyt*) show up as similar substances when photographed under phase contrast. Figure 12 as well as Figs. 3, 5, 6, and 9 are photographed with negative phase contrast (see Barer (3)).

Vesicle formation, which the writer believes is another important nuclear function, is shown best in Figs. 8, 9, 13, and 14. Figure 8 shows a vesicle (*ve*) inside a nucleus and a free vesicle (*rbc*) nearby. The color of both vesicles is orange, the free vesicle being brighter-colored than the intranuclear vesicle. In Fig. 9 the same two vesicles and the nucleus is photographed with negative phase contrast; here the free vesicle (*rbc*) shows the proper contour of a young red blood cell. The intranuclear vesicle (*ve*) shows a color tone which is more grey than the color tone of the young red blood cell. With May-Gründwald plus Giemsa stain it is usually possible to differentiate between "hemoglobin

vesicles" that are just being formed and the later stages that are red blood cells. The former are usually greyish orange, the latter clear orange. In Figs. 13 and 14 the large blast cell nuclei (*ma*, ma_1, ma_2) are seen at the moment at which they are releasing vesicles (ve_1, ve_2). In Fig. 13, the cell *eb* has the typical appearance of a classical erythroblast. Its nucleus looks "pycnotic" (it is in the contracted state) and the cytoplasm is basophilic. Mature red blood cells (*rbc*) are seen in both Figs. 13 and 14.

Discussion

It is obvious from the observations presented here and in earlier papers that the blood cells commonly dealt with in the literature have been selected because they possessed cytoplasm in various amounts. Only the lymphocyte was recognized early as a cell that often had no discernible cytoplasm. It was explained above how "cytoplasm" was formed from nucleoplasm during nuclear contractions; therefore blood cells regarded as typical would be cases where the nuclei were in a contracted state.

One must distinguish between nuclear contractions of the kind just described (see also p. 721 (5, 1956)) and the nuclear contractions which alternate with extensions and which are associated with intranuclear divisions of chromatin (basic nuclear units) reported, p. 715, Figs, 14, 15, (5, 1956).

The nuclear contractions dealt with in the present paper may leave the nucleus in the smaller (contracted) state and the newly formed cytoplasm can later be shed thus leaving a small naked nucleus. It is obvious then that here because of nuclear contraction we can have three different behavior stages which never would be associated in their proper sequence in fixed stained preparations. The first stage represents a large nucleus with very little cytoplasm, the second stage shows a small (contracted) nucleus surrounded by a broad rim of cytoplasm, and finally in the third stage the cytoplasm is gone and the small (contracted) nucleus remains. These three stages may only be 15 minutes apart such as they can be in cultures in vitro, yet in stained fixed preparations they would obviously be given three different cell names.

The great changes in nuclear morphology of blast cell nuclei described in this paper are believed to be particular stages of the blood cell life cycle.

The writer believes that the behavior of the basic nuclear units holds the key to the function of the lymphocyte and that the lymphocyte is the basic genetic element in the development of blood cells. Pape and Piringer-Kuchinka (12) believe small nuclei ("Kleinkerne") give rise to new lymphocytes. They observed a high rate of regeneration of normal lymphatic tissue in spleens of 39 rats after exposure to 1000 r. They believe that a form of amitosis (meroamitosis) has the greatest significance for cell regeneration. "Zahlreiche Meroamitotische Kleinkerne von ganz verchiedener Grösse und Form" are shown in their Fig. 7. The writer believes that the "Kleinkerne" correspond to one or more basic nuclear units, and so agrees that these small nuclei give rise to new lymphocytes. However, the term amitosis is not a good one to use, as the

development of the "Kleinkerne" or basic nuclear units is much more complex than a mere splitting of one nucleus into two. It is unfortunate that so many of us feel we must use the term "mitosis" whenever the nuclear federated autonomy displays its components in one way or another.

Hamilton (7), Trowell (15), and Hill (8) believe lymphocytic nucleoprotein is reutilized. The last two workers claim that the phagocytosis of pycnotic lymphocyte nuclei is a link in the cycle of reutilization. The present writer does not believe reutilization as such exists but that a proper life cycle is involved.

Yoffey (17, 18) believes that lymphocytes move into the bone marrow and form blood mother cells. The writer agrees that this is the only sound attitude to adopt. Yoffey *et al.* (19) have labelled lymphocytes of guinea pigs with tritiated thymidine. From the results of these experiments they conclude that the lymphoid tissue shows active synthesis of desoxyribonucleic acid. They also conclude that their results do not support the concept "either of massive reutilization or large scale recirculation".

Trowell (16), who wrote the chapter on the lymphocyte in a recent edition of the International Review of Cytology, puts the phenomenon described by Pape and Piringer-Kuchinka (12) under the subheading Death and Autolysis. He ends the chapter on the lymphocyte with a repetition of a statement made by Rich in 1936: "The complete ignorance of the function of this cell is one of the most humiliating and disgraceful gaps in medical knowledge." In the writer's opinion it is most unfortunate that the biological phenomena repeated over and over again by healthy normal blood cells and especially lymphocytes during their long and intricate life history are constantly being regarded as a sign of "pycnosis" and "karyorrhexis" and discarded by most medical research workers as a state of cell death. Consequently the biological function of the lymphocyte remains unknown to very many workers. Basic biological research is needed more than ever to clarify the role and developmental history of lymphocytes and other blood cells.

In addition to the role of the lymphocyte as a blood mother cell, it also appears to play an active part in other somatic tissues.

During the last 15 or more years the lymphocytic migrations into epithelium has been investigated by Andrew and Andrew (2). These workers believe the lymphocyte plays a part in the replacement of epithelial tissues. On the basis of observations of migrating lymphocytes and their behavior in epithelial tissue the writer believes the Andrews are describing one of the normal functions of lymphocytes.

Kelsall and Crabb (9) believe that the small lymphocytes synthesize, store, and transport nucleoproteins for use by other cells. During the many phases in the life history of lymphocytes and through the products produced during this time, this no doubt takes place.

Morishita (11) believes lymphoid elements are produced by extrusion of materials from the nucleated erythrocyte in the toad. The writer has worked with nucleated erythrocytes of birds, amphibians, and fishes for some time

(Engelbert, unpublished). From this work it has become increasingly clear that the nucleus of the red cell, at least during its young stages, behaves as a mother cell nucleus, which extrudes basic nuclear units. Morishita's claim seems therefore very reasonable. The nucleated red cell develops from a lymphoid cell. The nucelus of the lymphoid cell contracts gradually and the "hemoglobin vesicle" forms around it. In these forms the hemoglobin vesicle remains with the mother cell nucleus instead of being extruded as in the mammals.

Allfrey (1) reports that isolated avian reticulocytes are able to synthesize both heme and globin in vitro.

Normal mitosis of mammalian blood cells, which is taken for granted in textbooks and in the literature, has not been satisfactorily demonstrated (5, 6). Illustrations labelled "mitosis" are hardly ever convincing. Cronkite *et al.* (4) present an illustration (Fig. 3A) of "mitoses in normal bone marrow", which is similar to Fig. 27 in one of the writers papers (5). Figure 27 (5) shows a group of "giant chromosomes" from normal rabbit spleen. The "giant chromosomes" lie in a group without any visible sign of a cell boundary surrounding them. The writer believes now as then that the "giant chromosomes" represent one behavior phase of the basic nuclear units and that in this stage they are similar to "polytene chromosomes". In Cronkite's Fig. 3A the "chromosomal" elements are in a slightly more contracted state than in the figure of "giant chromosomes" referred to above.

Cronkite *et al.* used H^3-thymidine incorporation into DNA of blood cells in man and mice to study dynamics of hemopoietic proliferation. In Fig. 3B these workers show "labeled and unlabeled mitosis". The small bodies which presumably represent "mitosis" are very different from the ones presented in their Fig. 3A of normal mitoses. The difference in "grain counts" on nuclei in Fig. 4A–D does not tell us anything about normal mitosis in these cells, even if it shows active synthesis of DNA. The cell exhibiting the high number of grains could be a fusion product of cells, similar to the ones exhibiting half the number of grains. We have no real proof that a division has occurred. On the other hand blood cells are capable of extruding small or large particles within their borders very quickly and one wonders what significance the free grains which are seen in Fig. 4A–B and Fig. 5B–N have in relation to the history of the cell turnover. Although Cronkite's work does not help us to find evidence for a typical mitotic cycle it helps us in many other ways. Cronkite *et al.* uses the term primitive proliferating pool of cells (PPP) to avoid the many terms used for cells with similar functions. It is also very gratifying that these authors find that certain cells (basket cells), which in blood smear preparations commonly are termed degenerating, are actively synthesizing DNA and therefore are taken not to be "senescent degenerating cells".

J. H. Morrison and G. B. Wilson from the Department of Botany, Michigan State College, reported in 1958 in a private communication to the writer that they had treated spleens of 3-day-old rats with the "Feulgen squash

method". They reported absence of anaphases and believed that metaphase chromosomes passed directly into the telophasic state shortly to form band-shaped nuclei of young neutrophilic leucocytes.

In this laboratory we have prepared spleens of newborn mice with the Feulgen method and squash technique. The very brilliantly colored "chromosomal" bodies which are found in very large numbers in configurations similar to metaphases are not, in the writer's opinion, typical metaphases. Further, configurations similar to anaphases are never seen. The chromosomal configuration similar to metaphase separates out into three, four, or more chromosomal groups, which remain connected and later form ring- or band-shaped nuclei, taken to be young stages of polymorphic leucocytes.

Ste. Marie and Leblond (13, 14) studied mitosis in the thymus of young rats and reported high mitotic index of reticular cells and large, medium, and small lymphocytes. They show one illustration, Fig. 2 (14), of a typical anaphase in a reticular cell. Reticular cells and other mesenchymal elements, which form a source of embryonic connective tissue throughout the animal body, constitute a group of cells where normal mitosis is relatively easy to demonstrate. However, we still need to know how blood cells are formed.

Chromosome configurations observed by the writer (5) in mammalian blood cells ranged from tiny thread-like structures and granules to "giant" polytene chromosomes. Only in the small thread-like and the granular chromosomes could all mitotic stages be followed through. Such divisions have also been followed in in vitro cultures (Engelbert, unpublished). The dividing nuclei were small, of a size range usually found as basic nuclear units.

Acknowledgments

The researches on blood cell life history are supported by grants from the Ontario Research Council, the University of Toronto, and the National Research Council, Ottawa. Sincere gratitude is extended for these grants.

It is a pleasure to acknowledge the help of Dr. D. B. McMillan, who provided the preparations for Figs. 3, 4, 5, 8, 9, 11, and 12, and Miss V. V. Mileiko, who processed the photographs.

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SOME RADIOGRAPHIC OBSERVATIONS ON THE GASTRO-INTESTINAL AND URINARY SYSTEMS OF ANESTHETIZED PACIFIC SALMON (ONCORHYNCHUS)[1]

GORDON R. BELL AND JACK E. BATEMAN[2]

Abstract

Standard radiography with barium sulphate as the opaque medium was used to show the gastrointestinal and urinary systems of some intact, living *Oncorhynchus* species. The fish were anesthetized and the barium introduced into the systems by means of a syringe fitted with fine polyethylene tubing. Lateral and ventral views are presented in a series of photographs.

Introduction

There have been no published, detailed anatomic studies of *Oncorhynchus* species since the pioneer work of Green (1), despite years of intensive research on these fishes, and apparently no one has attempted to use radiography to study non-osseous structures in these fish. The advantages of studying functional rather than post-mortem anatomy are manifold. Radiography offers one of the most convenient methods of elucidating functional anatomy.

This paper, the outcome of a short investigation designed to clarify the shape and position of structures involved in proposed physiological studies, was considered of interest because of the paucity of appropriate anatomic data concerning Pacific salmon. Radiographic techniques will doubtless be fruitful in revealing many other anatomic features and functions in both intact fish and in excised tissues. The work of Mott (2) on the circulatory system of the eel elegantly illustrates this potential. It is hoped that this report will serve to demonstrate a useful technique and to stimulate further investigation of the anatomy of *Oncorhynchus* species.

Materials and Methods

In general, routine radiographic methods were employed but the $BaSO_4$ suspension used as the opaque medium was made very finely divided by precipitation from $BaCl_2$ with H_2SO_4. The precipitate was then washed repeatedly with water and stored in water. A flexible, opaque probe made by filling polyethylene tubing (0.011 in. I.D. × 0.024 in. O.D.) with barium sulphate suspension and sealing off both ends in a flame was used for preliminary radiographic examinations.

Exposures were made at approximately 55 kv and 40 milliampere-seconds using medical No-Screen film and a diagnostic X-ray unit.

[1]Manuscript received September 24, 1959.
Contribution from the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.
[2]221 Bastion Street, Nanaimo, B.C.

Gastrointestinal Radiography

Two-year-old sockeye (*O. nerka*) and coho (*O. kisutch*) salmon which had not been fed for 48 hours prior to treatment were anesthetized in a 1:20,000 solution of tricaine methanesulphonate (MS-222, Sandoz Pharmaceutical Co.), and barium sulphate was forced into the stomach by means of a 10-ml syringe fitted with an adapter and polyethylene tubing (Clay-Adams Inc.) until excess barium appeared in the mouth. The fish were then returned to water for various periods and anesthetized again before X-ray examination.

Urinary Radiography

Barium sulphate was introduced into the urinary tract by carefully working fine polyethylene tubing attached to a syringe up the posterior opening of the urogenital papilla while the anesthetized fish lay on its right side. If the genital system were entered, the tubing could be felt to curve sharply toward the head and follow the length of the body but if the urinary system were entered, the tubing curved only slightly toward the head and was directed dorsally. Once in the urinary system the tubing was worked gently along the duct until it would go no further, then the barium sulphate was injected slowly while the tubing was gradually withdrawn. Care should be taken to round the edges of the tubing, otherwise they may tend to cut fine structures.

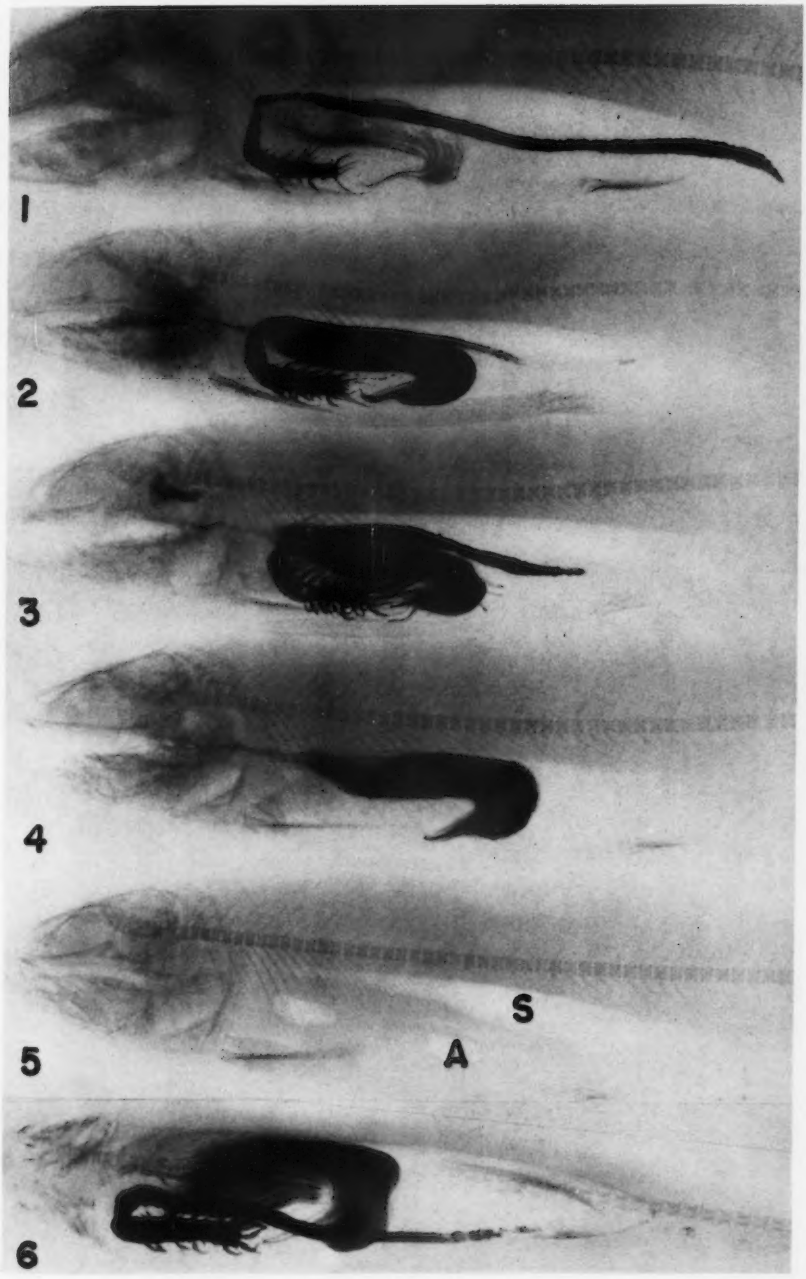
Results and Discussion

Gastrointestinal System

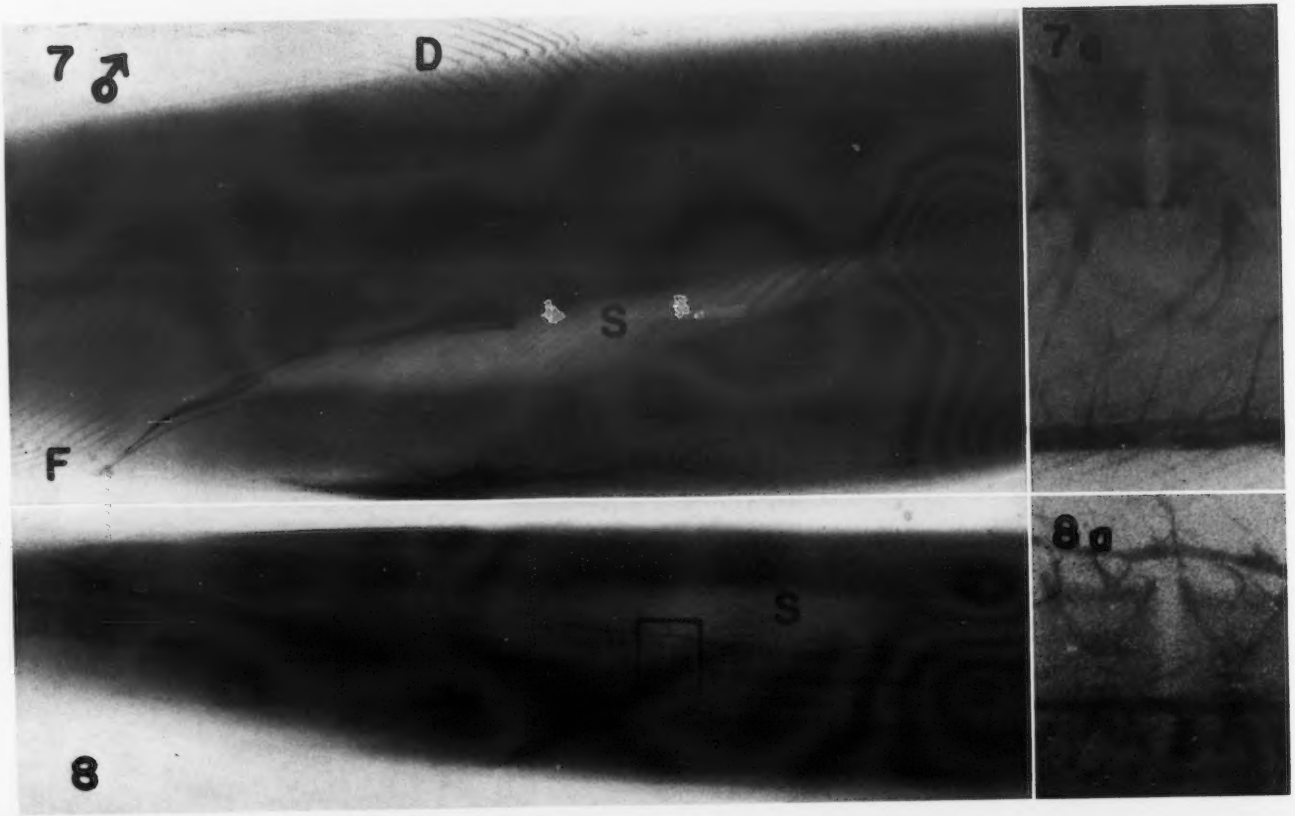
The results of the forced ingestion of barium sulphate by several fish are shown in Figs. 1–6 where the position of the barium is revealed by the very dark areas not present in Fig. 5, the untreated fish. In Fig. 1 it can be seen that some of the barium has passed completely through the gastrointestinal system and some has been trapped in the folds of the stomach musculature. The irregularities of the intestinal mucosa are also visible. Figures 1 and 2 show most clearly the constriction of the lower outlet of the stomach which might be termed the pyloric sphincter. In Fig. 2 some barium is seen still adhering to the mucosa of the lower intestine. The pyloric caeca, which help to increase the absorptive area of the otherwise short intestine, are shown particularly well in Fig. 3. It would be interesting to know how the food is passed through and out of these blind tubes. The shape of the stomach and the position of the esophagus are best illustrated in Fig. 4. Figure 6 helps to complete a three-dimensional "picture" of the gastrointestinal system but it is well to bear in mind that the anatomy of the system is modified

FIGS. 1–6. Radiographs of several anesthetized sockeye salmon (*O. nerka*) following the forced feeding of barium sulphate. The position of the barium in the gastrointestinal system is shown by the intensely dark areas.
FIG. 1. About 5½ hours after feeding.
FIG. 2. About 5 hours after feeding.
FIG. 3. About 3 hours after feeding.
FIG. 4. About ½ hour after feeding.
FIG. 5. Untreated fish.
FIG. 6. Ventral view at about 5 hours after feeding.
ABBREVIATIONS: S, swim bladder; A, probably ingested air.

PLATE I



Bell and Bateman—Can. J. Zool.



Bell and Bateman—Can. J. Zool.

according to the position of the body, as is the case in humans. However, it can be seen from Figs. 1, 2, 3, and 6 that the stomach tends to lie sinistrally, then loops anterodextrally and ventrally so that the loop of the intestine having the pyloric caeca lies dextrally. The caecal portion of the intestine then loops posterodorsally and the straight intestine continues dextrally to the anus.

Using the above technique in conjunction with fluoroscopy, it also may be possible to study peristalsis and residence times of food in parts of the gastrointestinal system while the fish are being held in retaining apparatus such as that described by Mott (2). In this regard, the behavior of mature, nonfeeding salmon would be of interest.

Urinary System

Parker (3) showed that in the brown trout (*S. fario*) the urinary and genital systems are entirely separate, thus correcting the common misconception that the two join posteriorly to form a single duct. According to our radiographic observations (Fig. 7) and those following latex injection of the two systems, this also appears to be true in both male and female coho salmon and is probably so in all *Oncorhynchus* species. In Fig. 7 the probe is shown in a sperm duct and the free barium in the urinary ducts. The kidney (or fused kidneys?) lies just ventral to the spine and runs the length of the body cavity and beyond to the pharyngeal region. Figures 7 and 8 show two mesonephric ducts with collecting tubules (Figs. 7*a* and 8*a*), which unite posteriorly and drain into a widening of the duct. The duct then continues posteroventrally and along the right side of the body cavity to terminate in the urogenital papilla posterior to the anus. The widening of the duct might be termed a bladder but its urine-storing function, with sphincter control, must first be established. The storage of urine by fishes might at first appear useless but it is possible that they need at times to withhold urine for resorption, or even in order not to attract predators.

It is remarkable that the barium penetrated the fine structures of this system but it is also possible that the opaque medium did not enter some significant ducts. "Miokon" and "Diodrast" which are used for urinary radiography in humans have so far been unsatisfactory for this purpose in salmon largely because there was much leakage from the site of injection, viz., the heart.

FIGS. 7 and 8. Radiographs of troll-caught coho (*O. kisutch*) 'grilse' following the injection of barium sulphate into the urinary system.

FIG. 7. The fine line of the barium-filled polyethylene tubing can be seen as the tubing lies in the lower end of the genital system, just ventral to the posterior end of the swim bladder. The free barium in the urinary ducts shows as dark areas and lines beginning posterodorsally to the barium probe and extending along the ventral surface of the kidney but apparently not into the head kidney region.

FIG. 7*a*. Photographic enlargement of area designated in Fig 7.

FIG. 8. The same fish as in Fig. 7 shown dorsally. Here the two mesonephric ducts with their branches appear as fine lines underneath and to the side of the spine. The fish is lying slightly to the right of vertical.

FIG. 8*a*. Photographic enlargement of area outlined in Fig. 8.

ABBREVIATIONS: S, swim bladder; F, anal fin; D, dorsal fin.

Summary and Conclusions

The gastrointestinal and urinary systems of intact, living sockeye (*O. nerka*) and coho (*O. kisutch*) salmon respectively have been examined by radiographic techniques. The position of the gastrointestinal system, musculature of the stomach, and probable existence of a pyloric sphincter are shown. Mesonephric ducts to the kidney are also shown, as well as a widening in the common duct, perhaps describable as a bladder if this function can be established. It is probable that the urinary and genital systems of *Oncorhynchus* species are separate; however, all species have yet to be examined.

Acknowledgments

We are very grateful to Drs. Bonnell, Roberts, Edmison, and Hacking for the use of their X-ray facilities. We would like to thank Dr. J. R. Brett for valuable advice and criticism concerning the manuscript.

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CHROMOSOME ABERRATIONS IN IRRADIATED CELLS OF CHINESE HAMSTER GROWN IN TISSUE CULTURE[1]

RESA WAKONIG[2] AND D. K. FORD[3]

Abstract

Various types of chromosome aberrations were described and their incidence recorded from analyses of metaphases derived from irradiated tissue cultures of the Chinese hamster. The aberrations included: chromatid breaks, incomplete breaks, isolocus breaks, various types of chromatid interchanges, chromatid intrachanges, minutes, and rings. The chromosomes taking part in various configurations could usually be identified, at least into certain groups. The aberrations encountered after irradiation were of the chromatid type. The lowest dose used was 15 rads and it caused abnormalities. The graph relating the incidence of breaks to the chromosome length was not a straight line but curved suggesting a nonproportionally large increase of breaks with the long chromosomes.

The advantages of the tissue culture technique and the unusual chromosome complement of the Chinese hamster were found to be, as anticipated, of great value in this study and could be utilized in various research problems.

Introduction

A number of recent reports are concerned with the action of X radiation on mammalian cells grown in tissue culture (2, 8). Most of the mammalian species have a high chromosome number and the individual chromosomes are hard to distinguish morphologically. The chromosome number of the Chinese hamster, *Criteculus griseus*, is low ($2n=22$), and each chromosome pair is recognizable by its size and position of the centromere (4, 11). The tissue culture technique and the characteristic complement were utilized in the present study. The incidence of abnormal metaphases caused by X radiation was recorded and the chromosome aberrations described.

Methods

The cell strain employed was the V-2 subline, the chromosome complement of which was recently described (3). When the experiments were started the cultures had 95% "diploid" cells. Forty per cent of the cells had the normal diploid pattern of 22 chromosomes, and 55% had 23 or 24 chromosomes. These hyperdiploid numbers are explained by the fact that the number and the morphological characteristics of the chromosomes may change any time in tissue culture, as described earlier (3, 4). Figure 1 demonstrates a typical example of a V-2 subline metaphase. Only "diploid" metaphases were studied in detail. The tissue culture methods were similar to those described previously (4) with the exception that the cultures were grown in Petri dishes in an atmosphere with controlled CO_2 content.

[1]Manuscript received July 21, 1959.
Contribution from Saskatchewan Cancer and Medical Research Institute, University of Saskatchewan, Saskatoon, Sask., Canada, and G. F. Strong Laboratory for Medical Research, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada.
[2]University of Saskatchewan.
[3]G. F. Strong Laboratory for Medical Research, University of British Columbia.

The radiations were performed with a 280-kv and 20-ma X-ray machine using 0.85-mm Sn, 0.25-mm Cu, and 1.0-mm Al filters. The dose rate was 50 to 60 rads/min. The irradiation was given 48 hours after subculturing. Then the medium was immediately changed and the cultures reincubated at 36–37° C. Since the yield of various chromosome aberrations is affected by the incubation time after radiation, the cultures were fixed at different time intervals: 12, 24, 36, and 48 hours. Before the cells were fixed and stained, they were pretreated with colchicine and hypotonic Hanks solution.

Results and Discussion

In the preliminary studies with samples derived from cell populations (stock V-2 subline) the incidence of abnormal metaphases generally increased with the radiation dose. There was a great increase at the dose of 30 rads, although the incidence varied in different cultures. For instance the exceptionally high incidence of 22 aberrant metaphases was recorded in 102 metaphases from a culture irradiated with 30 rads. With 15 rads there were some incomplete breaks and a few chromatid breaks. Any statistics of the incomplete breaks are only an approximation because there is a continuous variation from imperceptibly small lesions to large ones. At the extreme, a large lesion appears in the chromatid, the parts of which are joined together by a thread (as in Fig. 9). A small one appears as a slight bilateral constriction or indentation in the chromatid. The incomplete breaks are of importance as they are weak points in the chromosomes which might break later on (1).

With 30 rads all the cultures had numerous breaks. Usually only one of the chromatids was broken. Such a case is illustrated in Fig. 9.

With still higher doses there was an increasing number of breaks involving both chromatids. Sometimes the broken chromatid ends underwent sister reunion (Fig. 8). The first chromatid interchanges appeared with 90 rads.

To increase the uniformity of the results, cultures derived from single cells (a V-2 clone) with usually 23 and rarely 24 chromosomes were irradiated with 180 rads. Some of the chromosome aberrations are illustrated in Figs. 3 to 11. The incidence of the most important abnormalities are given in Tables I and II.

Table I gives the incidence of abnormal metaphases and of the various types of gross abnormalities found in cells 12 hours after radiation as contrasted to cells not irradiated. Three different types of chromatid interchanges are illustrated in Figs. 3 to 7, 9, and 10, and three types of chromatid intrachanges in Figs. 8, 9, and 11. The group "others" of Table I includes chromatid interchanges between more than two chromosomes (Fig. 7). The different types of chromatid exchanges have been described and their underlying mechanism discussed by Revell (9) and Lea (6).

In about half of the chromatid interchanges it was possible to recognize the chromosomes involved. Identification of the individual chromosomes would have been considerably easier if the chromosome complement had been normal instead of hyperdiploid. Those chromosomes that were not individually recognized could nearly always be recognized in three different groups

PLATE I

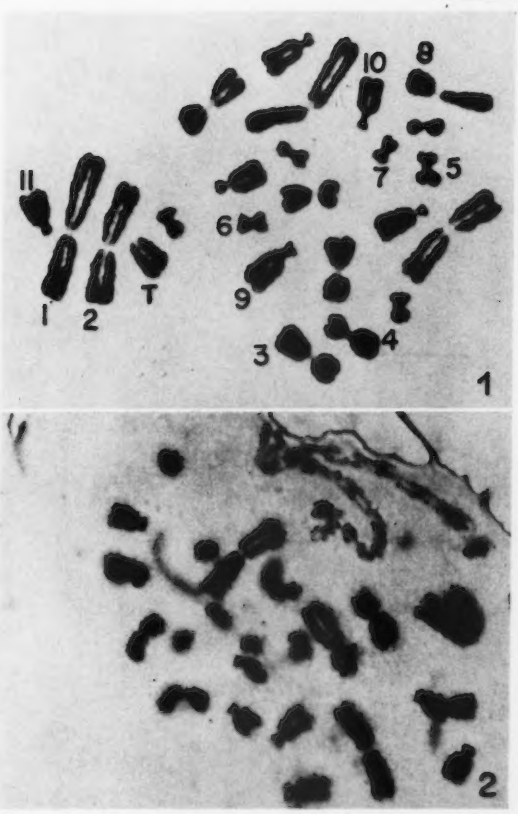

FIG. 1. Hyperdiploid C metaphase after exposure to 20 rads; 23 chromosomes + 1 atypical telocentric; normal complement except with an extra chromosome (No. 6 or No. 7), with the telocentric chromosome, and with one of No. 8 missing; a haploid set of chromosomes is numbered; the telocentric chromosome is marked with T. × 2750

FIG. 2. C metaphase after exposure to 30 rads; No. 1 and No. 3 in a stage not synchronized with other chromosomes. × 2100

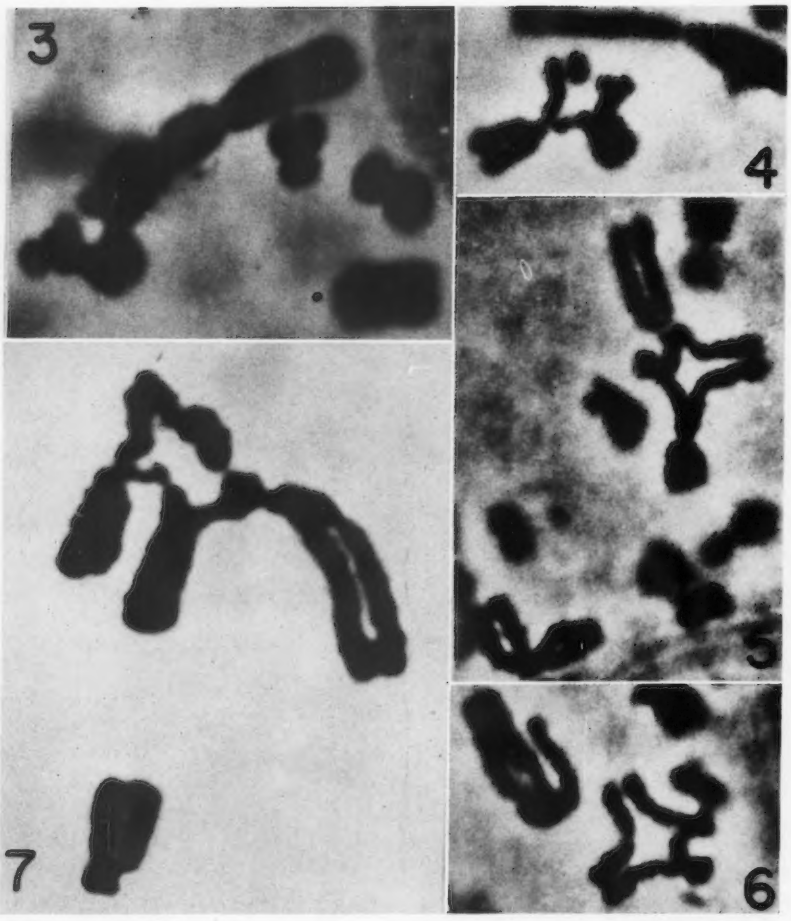

FIG. 3. Part of a cell at metaphase after exposure to 180 rads; triradial (No. 1 and No. 9 or No. 10). × 4500

FIG. 4. Part of a cell at metaphase after exposure to 180 rads; quadriradial (No. 8 and No. 9). × 3400

FIG. 5. Part of a cell at metaphase after exposure to 180 rads; quadriradial (No. 1 and No. 8). × 3400

FIG. 6. Part of a cell at metaphase after exposure to 180 rads; quadriradial between two homologous chromosomes (No. 9 and No. 9). × 3400

FIG. 7. Part of a cell at metaphase after exposure to 180 rads; pentaradial (No. 1, No. 3, and No. 8). × 4500

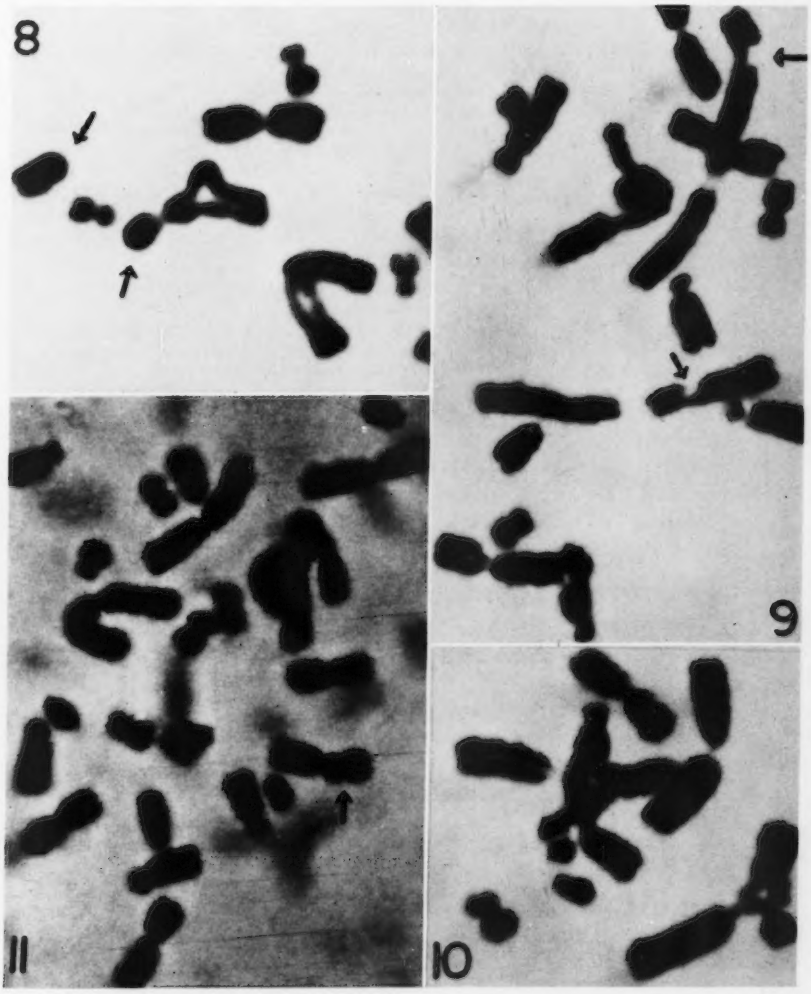

FIG. 8. Part of a cell at metaphase after exposure to 180 rads; chromatid intrachange consisting of a chromatid duplication and a deletion in one arm of No. 1; in the other arm an isolocus break with a sister reunion (arrow); the fragment shows a sister reunion (arrow). ×3400

FIG. 9. Part of a cell at metaphase after exposure to 180 rads; chromatid interchange (triradial) between No. 8 and No. 9 or No. 10; chromatid intrachange in No. 1 with a loop formation; chromatid break in No. 1 (arrow). ×3400

FIG. 10. Part of a cell at metaphase after exposure to 180 rads; two triradials (No. 1 and No. 3 or No. 4; No. 9 and one not identified because of an overlaying chromosome). ×3400

FIG. 11. C metaphase after exposure to 180 rads; chromatid intrachange with loop formation in No. 1; several incomplete breaks (arrow). ×2800

TABLE I

Percentage incidence of aberrant metaphases 12 hours after exposure to 180 rads (based on 100 control and 200 treated cells)

	Aberrant metaphases	Chromatid interchanges			Chromatid intra-changes	Rings
		Triradial	Quadriradial	Others		
Control	13*	0	0	0	0	0
Treatment	74†	11	9.5	2.5	13.5	2.5

*Nine per cent incomplete, 1% isolocus, 3% chromatid breaks, and 1% minutes.
†Different types of aberrations as shown in Table II.

TABLE II

Incidence of various aberrations in 60 randomly selected aberrant metaphases 12 hours after exposure to 180 rads

Type of aberration	% incidence in aberrant metaphases
Chromatid breaks	87
Incomplete breaks	180
Isolocus breaks	48
Chromatid interchanges	40
Triradials	18
Quadriradials	18
Others	4
Chromatid intrachanges	23
Minutes	57

each having two similar chromosomes. For instance in Fig. 3 the chromosomes No. 1 and No. 9 in the triradial configuration can be easily identified. Chromosome No. 1 is distinguished by its great length and metacentric position of the centromere and No. 9 by its intermediate length and acrocentric position of the centromere.

Out of 14 quadriradials encountered in this study 5 were between homologous chromosomes at homologous loci (Fig. 6). Although the data are few, this incidence is much higher than a randomly expected one, suggesting a tendency to somatic pairing.

Table II shows the incidence of all different types of aberrations recorded in 60 aberrant metaphases studied in great detail. Extra care was necessary for the accurate recording of chromatid breaks. Some of these are difficult to distinguish from incomplete chromatid breaks but an attempt was made to be consistent in separating these. A break was only scored when the dislocation of the broken chromatid ends was clearly visible. Two very obvious breaks are shown in Fig. 9 and less obvious ones in Fig. 11.

In a few cells almost all the chromosomes were broken and their identity lost. It is assumed that such cells happen to be in a very sensitive stage when irradiated. According to Sparrow (10) the chromosomes undergoing division are exceptionally sensitive to radiation.

Metaphases were also studied 24, 36, and 48 hours after radiation. The findings were qualitatively similar to those in Tables I and II. However, with increasing time-interval the incidence of aberrations decreased. This was also reported by Bender (2) for epithelioid kidney cells from man.

Nearly all the chromosome abnormalities encountered in this study were of the chromatid type. Probably most cells studied had been in late resting stage of the division cycle when irradiated. The data of Puck (7), in a study with a method similar to ours, also show mainly chromatid type of aberrations in euploid human cells.

It is interesting to consider whether the incidence of breaks in different chromosomes is in a linear relationship to the chromosome length. The data in Fig. 12 show that this was not the case in the cells studied. The increase in incidence of breaks with increasing chromosome length was much greater than in a linear ratio. An explanation to this is that, although the initial breaks may occur randomly per unit length in any chromosome, the restitution process may take place more readily in small chromosomes.

According to the curve in Fig. 12 more breaks occurred in long than in short chromosomes. Similarly the chromatid intrachanges and chromatid exchanges were more common in the longest chromosomes. Out of 17 intrachanges, 10 occurred in the 2 longest chromosomes, and out of 26 chromatid exchanges, 18 involved the 2 longest chromosomes.

In two cells irradiated with 30 rads and in one irradiated with 90 rads, a peculiar type of a chromosome was found. In metaphase this appeared as a poorly stained thread with a chromomere-like structure as is known for chromosomes in meiotic prophase (Fig. 2). The length of such a chromosome was about twice as long as in the normal metaphase chromosome. Such

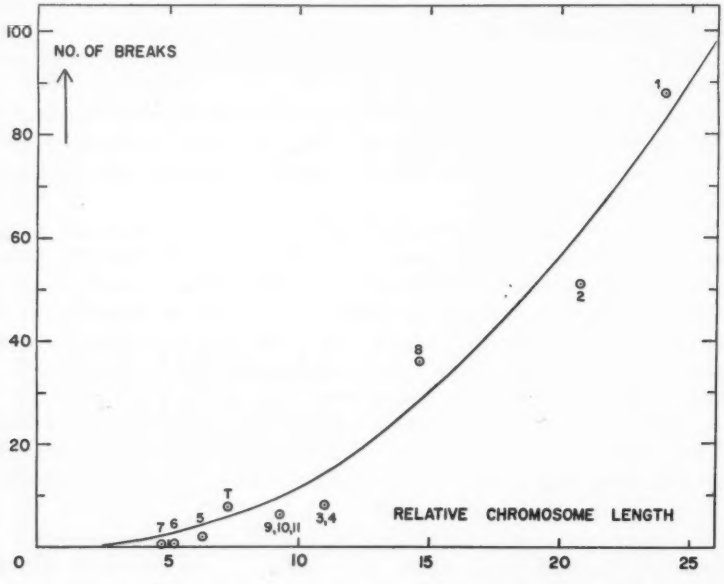

FIG. 12. Incidence of breaks in chromosomes of different lengths after exposure to 180 rads; for the numbers of the chromosomes at the curve see Fig. 1.

retarded chromosome development was found once in chromosome No. 1, once in No. 3, and twice in No. 9 (for the numbers see Fig. 1). The incidence of this abnormality was so low that it is impossible to conclude whether it was caused by radiation or was of spontaneous occurrence. Similar unspiralized chromosomes have also been reported in irradiated wheat by Jain (5).

Acknowledgments

The authors wish to thank Dr. Harold F. Batho for supervising the irradiation technique and Christina Boguszewski for technical assistance.

The investigation was supported by the National Cancer Institute of Canada, project No. 178, and by a special grant from the British Columbia Division of the Canadian Cancer Society.

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THE OLFACTORY APPARATUS OF PETROMYZON MARINUS L.[1]

H. KLEEREKOPER AND G. A. VAN ERKEL

Abstract

The olfactory organ of *Petromyzon marinus* was studied anatomically and histologically in adult and larval forms and compared with that of related cyclostomes as reported in the literature. The single olfactory organ of this species consists of three parts: the nasal tube, the olfactory sac, and a nasopharyngeal pouch. The tube is lined with indifferent epithelium and contains a valve at the entrance of the sac. The valve directs a jet stream of water into the sac when water penetrates the nasal tube. The structure of the valve allows it to reverse its position when water is expelled from the organ. The sac or nasal chamber is divided by 25 folds of its walls, 13 on the right, 12 on the left side. One of the folds, in median dorsoventral position, divides the sac into two chambers but for the anterior extremity of the sac. The folds are lined with epithelium which contains the olfactory cells. The latter are not distributed evenly in the epithelium but are limited to those areas of the folds which face a neighboring fold. The peaks and valleys of the folds are lined with indifferent epithelium. The connective tissue in the posterior and dorsoposterior regions of the sac contains clusters of cells within blood sinuses. These clusters are the accessory olfactory organ; no open communication could be found between the clusters of cells forming this organ and any other part of the olfactory organ. The organ is innervated by rami of the n. olfactorius which is formed by the sensory cells of the folds in the nasal sac. The whole is surrounded by the cartilaginous nasal capsule. The pouch is a continuation of the nasal tube in ventral and ventrocaudal direction and lies between the digestive tract and the nasal sac and brain. Its posterior part is enlarged laterally and ends as a blind pouch at the level of the second internal gill opening. Since the pouch is not protected by rigid cartilage it is subject to the rhythmic contractions and relaxations of the respiratory muscles and body walls, thus sucking in water during the inspiration and expelling it during the expiration phase.

Introduction

In 1958 a study of the chemical senses, particularly of olfaction, in *Petromyzon marinus* was initiated in this laboratory in connection with investigations on the mechanisms by which the parasitic species localizes its prey. Early in this work it became evident that olfaction plays an important rôle and extensive research has been in progress since, under the direction of the senior author, to elucidate some of the physiological, behavioral, and chemical aspects of olfaction in *P. marinus*. It also became desirable to gather information on the anatomy and histology of the olfactory organ in order to gain better understanding of its function, particularly since the olfactory apparatus is a most peculiar organ in the cyclostomes and especially in the Petromyzontidae. Initially it was assumed that this information could be obtained fairly easily by a study of the pertinent literature. It soon became evident, however, that in spite of a vast and widely dispersed literature on cyclostomes generally, very little pertinent information was available on the structure of the nasal apparatus in *P. marinus*, the species with which our physiological investigation was concerned. Much of the literature does not refer to the identity of the species studied while another part deals exclusively with such

[1]Manuscript received July 14, 1959.
Contribution from the Department of Biology, McMaster University, Hamilton, Ontario.

common European species as *Lampetra fluviatilis* and *L. planeri*. The former is parasitic, the latter non-parasitic. Specific information on *P. marinus* is very rare indeed. It was, therefore, decided to make a general study of the anatomy and histology of the olfactory organ of the species. This paper describes the organ and its parts in the adult and in the ammocoete and presents some of the findings by other authors who have studied the nasal apparatus in petromyzonts. Only exceptionally could specific reference be found to *P. marinus*. Although the study of the literature was extensive, some obscure but pertinent papers may have escaped our attention. The description of the olfactory organ in this paper is based on observations on four larvae (41, 71, 82, and 110 mm long) and two adults (135 and 300 mm long) collected in the Lake Huron and Lake Michigan basins.

This study was made possible by the support received from the Research Division of the Department of Lands and Forests of the Province of Ontario, which is here gratefully acknowledged. We also thank Dr. V. C. Applegate of the Hammond Bay laboratory of the U.S. Fish and Wildlife Service, who supplied us with some of the lamprey material required for this study.

General Description of the Organ

The general morphology of the olfactory apparatus of *Petromyzon marinus* is similar to that of other Petromyzontidae and is represented by Figs. 1 and 2. These are photographs of a wax model based on microscopic preparations of 10 μ thickness of the olfactory organ in an adult animal of 30 cm length. The organ consists of three main parts: a nasal tube with a single nostril; a nasal sac containing the sensory part; and a pouch, referred to in the literature by a variety of names but which will be called in this paper the nasopharyngeal pouch, as proposed by Leach (33).

The nasal tube has a single external opening on the dorsal surface of the head in a median position just in front of the eyes in the metamorphosed animal. The skin around this opening forms a slight elevation which appears on the head as a nearly circular rim (Figs. 1 and 2, *a*). From this external opening the nasal tube slopes ventrocaudad, then bends into a straight caudal direction. The nasal sac lies caudad and dorsad of this bend (Fig. 1, *d*). The tube continues caudad below the nasal capsule, then bends ventrocaudad below the fore- and mid-brain, after which it resumes its straight caudal direction following the anterior and ventral contour of the cord. The posterior end of the tube widens laterally to a large pouch which is ventrodorsally compressed in the median plane (Fig. 1, *g*). While in the Myxinoidea this pouch connects with the gut, in the Petromyzontidae it ends blindly in the vicinity of the second branchial opening. Just before the entrance opening leading into the nasal sac, the wall of the tube forms a fold which functions as a valve (Fig. 3) and which is described below. The nasal sac connects with the nasal tube at the point mentioned (Fig. 3, *a*). In the adult, it is surrounded by a cartilaginous capsule from which two olfactory nerves protrude through two lateroposterior openings (Fig. 1, *e*) (56).

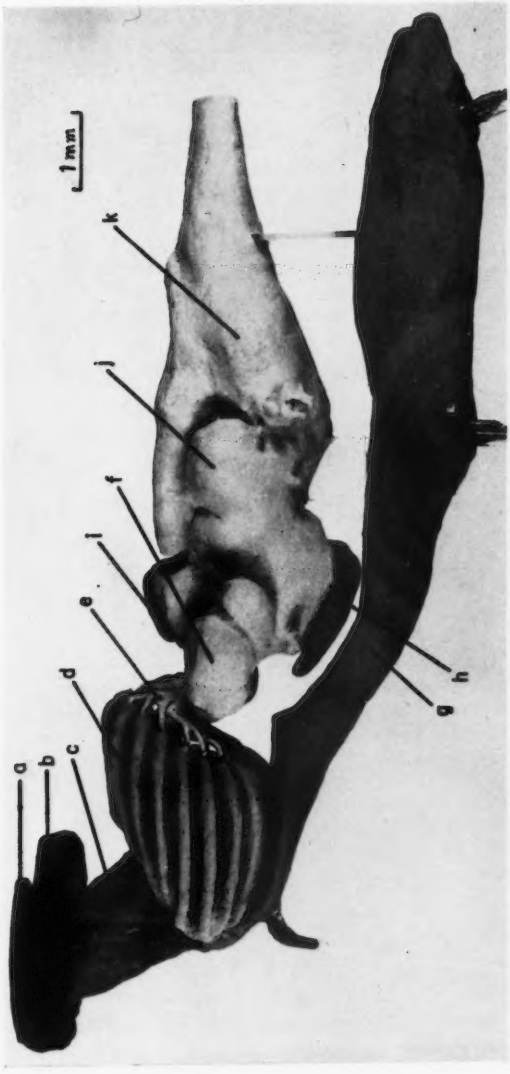

FIG. 1. Wax model of the olfactory organ and brain of *Petromyzon marinus* (30 cm). Side view. *a*, nostril; *b*, rim of nostril; *c*, nasal tube; *d*, folds of the mucosa in the nasal sac; *e*, olfactory nerve; *f*, bulbus olfactorius; *g*, nasopharyngeal pouch; *h*, pituitary gland; *i*, epiphysis; *j*, optic tectum; *k*, medulla.

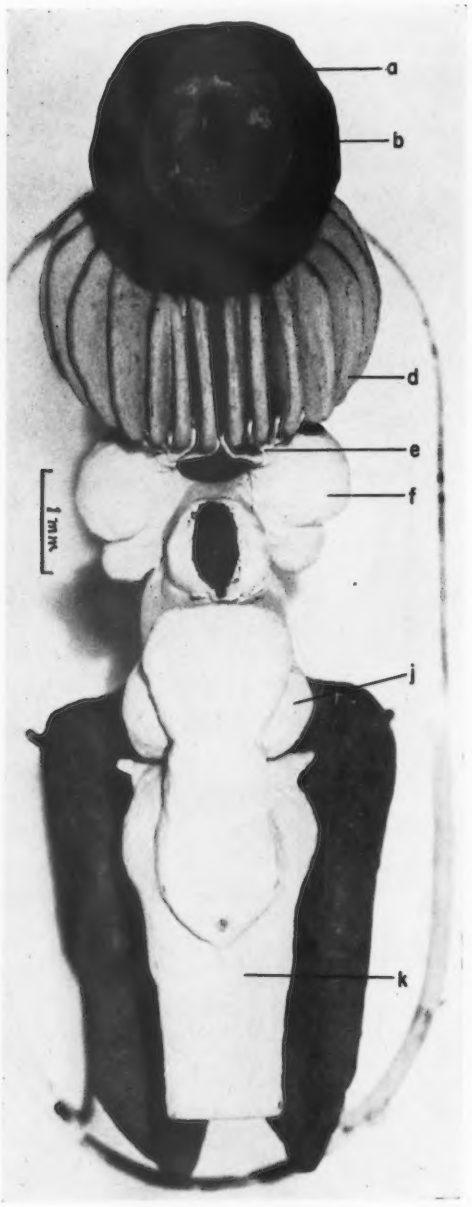

FIG. 2. Same as Fig. 1. Top view.

The nasal sac is divided in a number of flat chambers which have been called secondary nasal sacs (38) (Fig. 1, *d*). These chambers are formed by folds of the mucosa. One median fold is higher than the others and thus divides the sac into two halves. All folds carry both sensory and indifferent epithelium. Below and behind the nasal sac, the so-called accessory organ is found. Both the sac and the accessory organ are enclosed by a cartilaginous capsule which was omitted from the model represented in Figs. 1, 2, and 3 in order to show the disposition of the folds of the mucosa and the resulting chambers.

Development

The embryonic development of the apparatus was not the object of the present study but a few general remarks based on the literature on the development of cyclostomes will be useful for the understanding of some phylogenetic relationships. Further reference to the embryonic development of the three parts of the apparatus will be made in the detailed description of these parts in *P. marinus*.

Earlier observations to the contrary (10), the development of the olfactory organ in cyclostomes begins from a single olfactory placode (58, 59, 49) which is formed by a median thickening of the ectoderm at the ventral side of the head (30, 40, 39). This thickening and subsequent penetration of the ectoderm into the olfactory groove occurs at the locus where the brain finally separates from the ectoderm, i.e., at the neuropore. According to von Kuppfer (58, 59, 49) this pole of the brain is homologous with the lobus olfactorius in *Amphioxus* while the olfactory groove in the lamprey was believed to be homologous with the ciliated groove in *Amphioxus* by Kölliker (29). This implies that the ammocoete has a single lobus olfactorius during its early development (26). A similar single formation can be found in the gnathostomes; however, the paired olfactory placodes of the gnathostomes occur already in the larval lamprey in which they form, together with the olfactory groove, a single organ. There may thus not be a distinction in principle between monorhiny in lampreys and amphirhiny in gnathostomes (30, 36, 39). *Petromyzon* would thus be a transition form which in the course of its ontogeny shows both the olfactory groove of *Amphioxus* and the lateral olfactory placodes of the gnathostomes. This view has, however, not been accepted universally and some authors believe that monorhiny in the cyclostomes is a secondary development from primary amphirhiny (20, 34, 35).

The early development of cyclostomes relative to the relationships between the hypophysis and the nasal apparatus was referred to by Leach (33), who also reviewed the literature pertinent to this problem of old-standing. Leach points out that a nasopharyngeal stalk is formed at an early stage from somatic ectoderm in close association with the developing olfactory sac. From the caudal tip of this stalk, the pars intermedia is cut off while the pars anterior develops later from a dorsal thickening of the caudal portion of the remaining stalk. During later development in the ammocoete this pars detaches completely leaving a remnant of the nasohypophyseal stalk which persists

as the nasopharyngeal stalk. The latter attaches to the caudoventral aspect of the nasal sac and forms, during the metamorphosis of the larva, the nasopharyngeal pouch. The lumen of this pouch is formed by the ultimate joining of numerous cavities in the nasopharyngeal stalk which arise during the caudal elongation of the stalk in the course of larval growth. Eventually a continuous lumen is present from the caudal extremity of the pouch to the nasal sac (33).

Two primary nasal sacs are formed at an early stage by the olfactory groove which develops two lateral lobes separated by a fold. The sacs are completely lined with sensory epithelium at this early stage (46, 27). Subsequent folding of this epithelium leads to the formation of lamellae and of the "secondary sacs" (38, 21) and the development of regions of indifferent epithelium as described below. At a later stage the nasal epithelium forms diverticulae at its caudal and ventrocaudal aspects; the diverticulae are separated by subsequent abscissions. These so-called "follicular appendages" are referred to as the accessory organs which receive branches from the olfactorius (6, 25, 27, 38).

The Nostril

The median external opening of the nasal tube lies immediately in front of the epiphysis and is surrounded by normal epidermis which is thickened into a rim or ridge bordering the tube (Figs, 1, *c*; 2, *c*, *d*). The dorsal position of the nostril must be attributed to the development of the mouth and the very large upper lip which pushes the opening of the nasal sac towards the dorsal side (39, 14). In *Lampetra fluviatilis* the rim of the nostril contains touch cells, according to Fahrenholz (17). No such cells were found in the nostril of *P. marinus* (Fig. 4). Since the lumen of the nostril can be seen to vary synchronously with the phases of respiration, attention was given to the possible occurrence of muscle fibers in or around the rim. However, no muscle fibers were present in such position in any of the material studied. It seems likely that the constriction and dilation of the nostril results from the general contractions of the body walls in the course of respiration (13, 54).

The Nasal Tube

The nasal tube is lined with stratified epithelium of flattened cells, which have a cuticular cover at their free extremity and which are similar to those found in the epidermis of the skin. In the vicinity of the nostril this epithelium is profusely innervated, most likely by sensory nerves. However, the granulated and cup-shaped cells generally found in the epidermis are absent from the epithelium lining the nasal tube. This resembles the characteristics of the nasal tube of *L. fluviatilis* as described by Ballowitz (2) and Langerhans (31).

The structure of the epithelium in the ammocoete differs from that in the adult (Fig. 5). In the larva there is a single layer of high cylindrical ciliated cells. During the transformation of the ammocoete the cilia seem to disappear and in the adult the epithelium gains the characteristics mentioned above. Similar changes have been observed by Kaensche (28) and Schneider (55) in *L. planeri*. The function of the cilia in the ammocoete would be that of

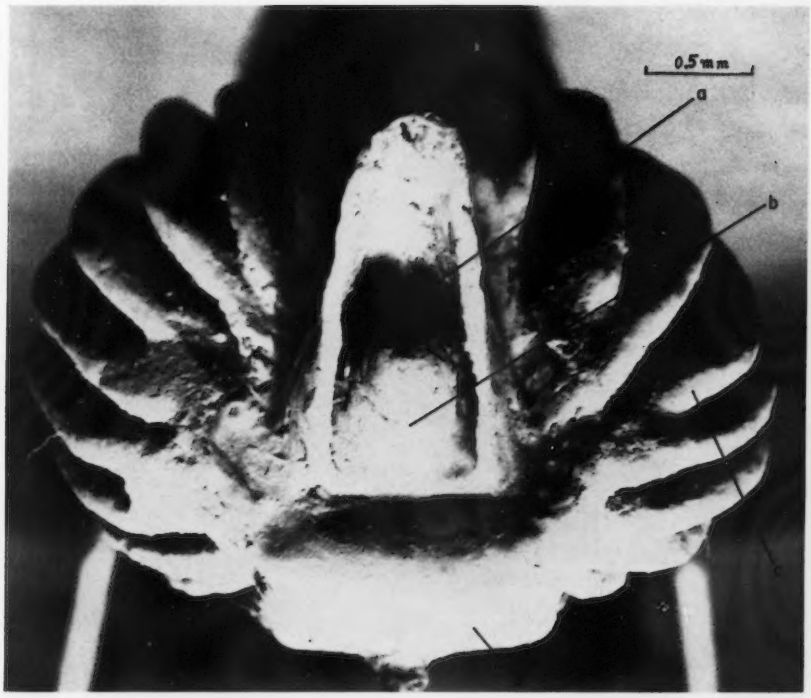

FIG. 3. Front view of the olfactory organ of *P. marinus* after removal of the nasal tube. *a*, entrance of nasal sac; *b*, valve in the position which it assumes during expiration; *c*, folds of nasal epithelium; *d*, nasopharyngeal pouch.

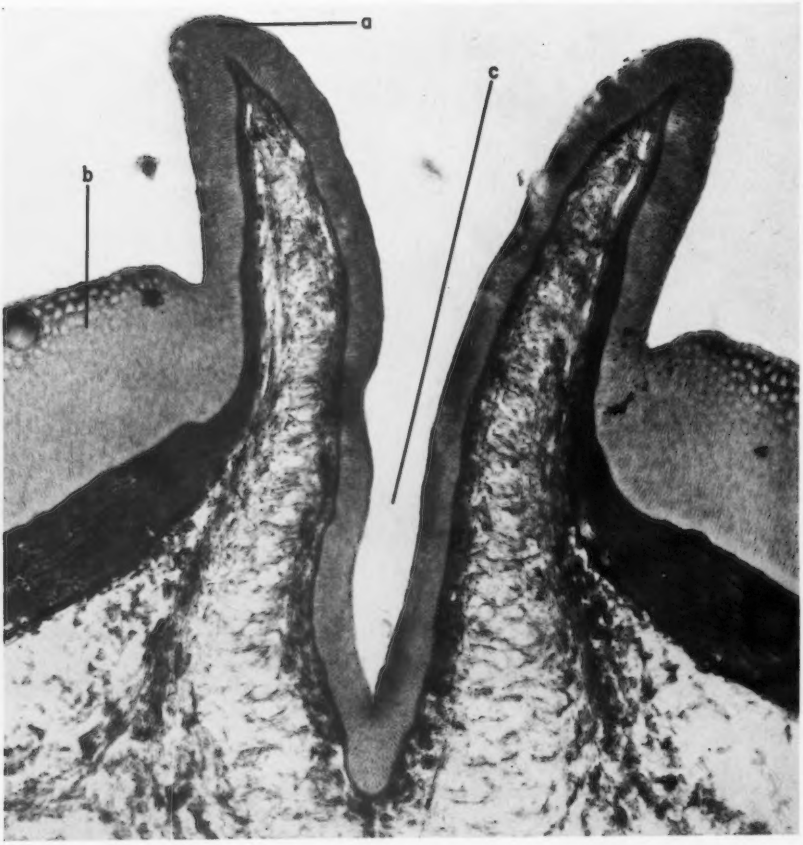

FIG. 4. Cross-section of the nostril of *P. marinus* (13 cm long). *a*, rim of nostril; *b*, skin; *c*, nasal tube.

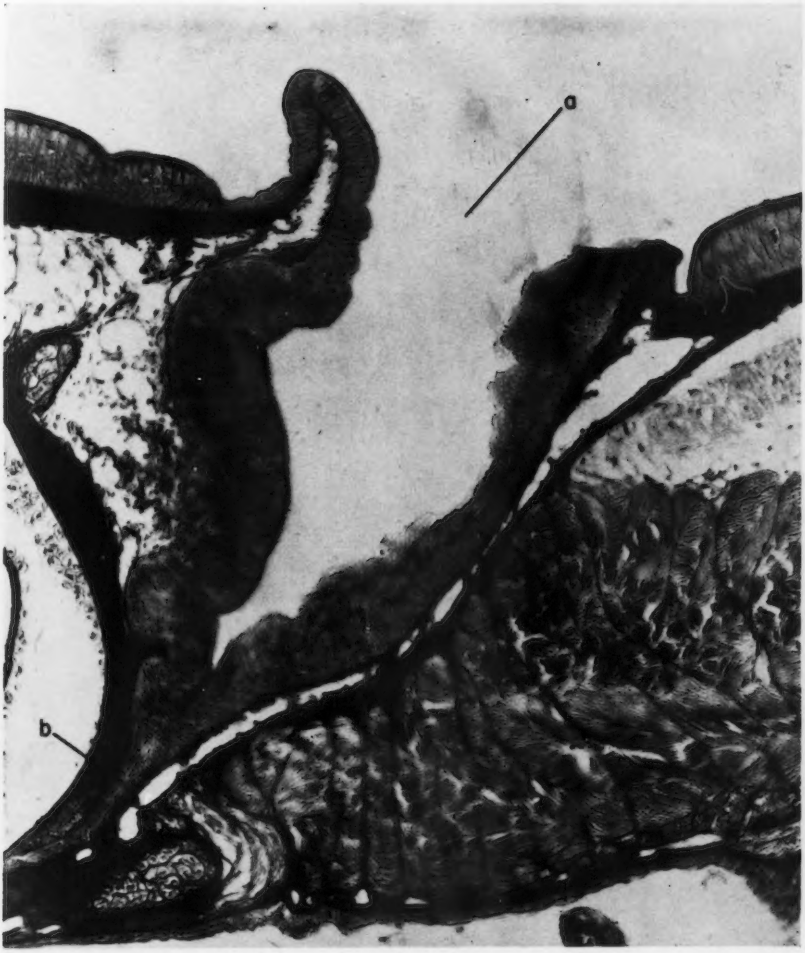

FIG. 5. Median section through the olfactory organ of a 37-mm larva of *P. marinus*. *a*, nasal pit; *b*, hypophyseal stalk.

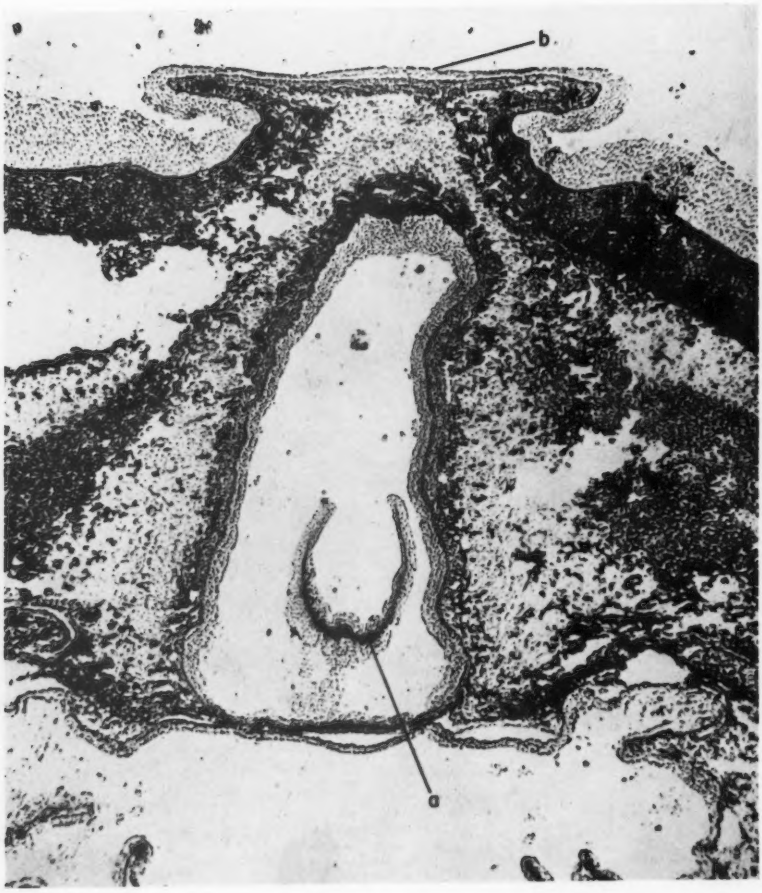

FIG. 6. Valve of a 30-cm long *P. marinus* in forward position (expiration period). *a*, valve; *b*, nostril.

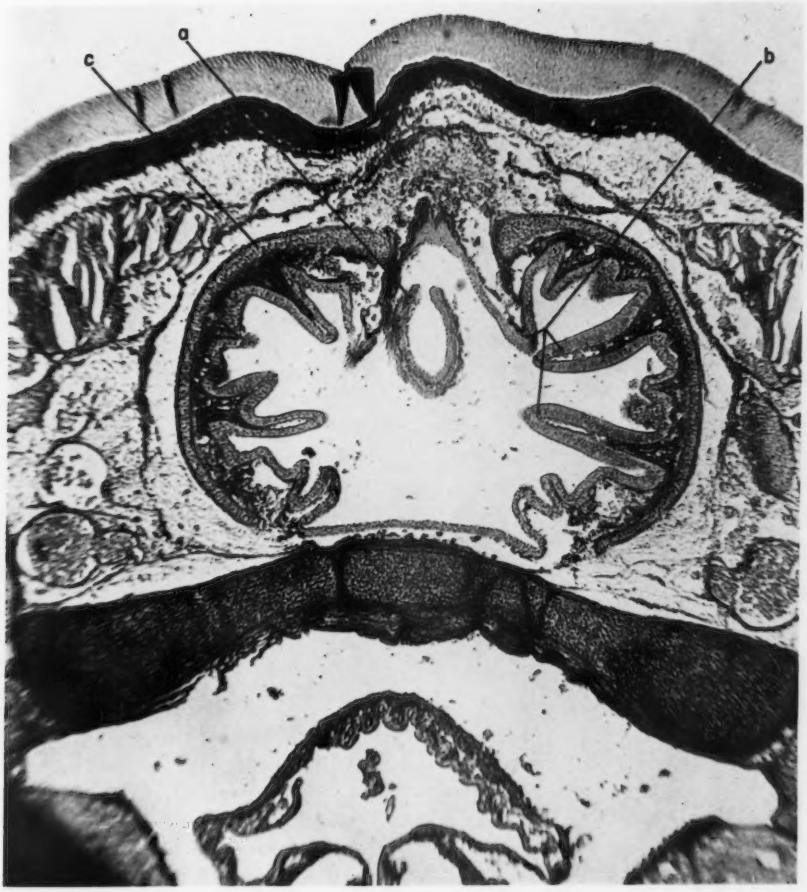

FIG. 7. Valve of 13-cm long lamprey in backward position (inspiration period). *a*, valve; *b*, folds of mucosa in nasal sac; *c*, nasal capsule.

impelling a current of water into the tube and to strain out mud particles (Schneider (55)). If this is indeed the function of the cilia, it might be assumed that the olfactory organ is functional in the larva. There is, however, no general agreement on the function of the cilia and of the olfactory organ in this stage of development (15). In the adult the nasal tube is surrounded by cartilage which is not found in the larva.

The Valve

Lateroventrally, at each side of the entrance of the nasal sac, the walls of the nasal tube develop an evagination. The two evaginations unite to form a fold which connects with the floor of the nasal tube in the manner shown in Figs. 3, 6, and 7. A transverse fold is thus formed which extends from the floor upward but leaving a lumen between the roof and the free upper edges of the evaginations. This lumen is the opening of the nasal sac. The attachment of the fold to the wall is oblique in relation to the long axis of the tube and the shape of the lumen varies with the position of the fold (Fig. 6). The fold is thin and flexible and can move backwards and forwards with the water current in the tube. Figures 6 and 7 show the positions of the fold in sections of two different lampreys. Figure 6 represents the fold in its most forward position inside the nasal tube; in Fig. 7 the valve has moved backwards into the olfactory sac.

A "valve" of similar structure was described by Imamura (27) in *L. planeri* and *L. fluviatilis*. In these forms, however, the length of the valve seems to be much smaller than that of the valve found in *P. marinus*. The function of the valve has been interpreted in various ways. Kaensche (28) believed it to be primarily a straining mechanism by which mud particles would be caught in the narrow corner formed by the valve and the roof of the tube during inspiration as a result of stretch on the walls of the valve. During expiration the walls would relax and the accumulated foreign matter expelled with the water. However, there seems to be little likelihood that the very flexible walls of the valve, which maintain an open slit at their free dorsal edges, could be stretched sufficiently to act as a strain for foreign matter introduced with the water (27). We believe that the function of the valve is that of directing the inspired water jetlike fashion into the nasal sac where the water must enter the narrow lumina separated by radially placed lamellae in order to reach the olfactory epithelium. The action of the valve is entirely passive since muscle fibers are absent from its structure. Vogt and Yung (69) believe that the valve impedes the entrance of water into the nasopharyngeal tube during inspiration, providing free passage outwards during expiration. It is likely that the entrance of water into the nasopharyngeal tube is somewhat hindered during inspiration but the position of the valve could not prevent the passage of water altogether. Were this so the nasopharyngeal pouch could not fill with water and would lose much of its pumping efficiency (see below).

Nasal Sac

The nasal sac in lampreys consists of a number of chambers separated by radially oriented lamellae of connective tissue arising from the walls of the

sac (Fig. 8). Centrally the edges of the lamellae are free so that a central lumen remains. The anterior and posterior extremities of the lamellae coalesce thereby narrowing the lumina between them to form fine tubes (Fig. 9). One of the lamellae forms a dorsoventral septum in the median plane thus dividing the posterior portion of the sac into two chambers which are slightly asymmetrical in *P. marinus* (Fig. 8, *c*). In the anterior portion, towards the opening of the sac into the nasal tube, this median lamella is absent so that this part of the sac consists of a single chamber.

The walls of the lamellae are covered with sensory and indifferent epithelium. The sensory epithelium on each lamella is limited to the walls which face the neighboring lamellae; it is absent from the tip of the lamella facing the central lumen and from the base lying between two lamellae (Fig. 10) where it is replaced by indifferent epithelium (Fig. 10, *c*).

The sensory epithelium (Fig. 10) consists of two forms of cells (53, 50, 57, 18, 16, 14): (1) *Supporting cells.* These are long rodlike or cylindrical cells which extend through the whole thickness of the epithelium and whose cytoplasm is clear or only little granulated. The nuclei of these cells lie in the lower half of the body. In their vicinity Retzius observed yellow-pigmented granules. The free extremity of the cells is squarish in top view and is covered with thin ondular cilia (16). This ciliated cover has also been described in *L. fluviatilis* (31, 18). The cell base is frequently widened and rests on the connective tissue of the lamella. (2) *Sensory cells.* They have a shorter body than the supporting cells so that their base does not reach the lower limit of the epithelium. The nucleus is located higher in the cell body which has an elongated pear-shape with the wider extremity at the free surface of the epithelium. The lower extremity has a pointed and curved extension which continues into nerve fiber. The cytoplasm of these cells contains many granules but apparently lacks the yellow bodies described by Retzius (53). The sensory cells, which have an irregular angular cross-section, are usually separated by the more numerous supporting cells (50); where sensory cells touch each other they do so always at an angular point and never along their large faces (3, 37). The free surface of these cells seems to carry short stiff cilia in *L. planeri* (4). Such cilia are not evident in adult *P. marinus* although they could be demonstrated by Retzius (52) in a single case. In the ammocoete the sensory cells carry a distinct brush of short cilia (52).

To obtain an estimate of the total number of sensory cells in the adult lamprey the surface of the olfactory folds was measured on the wax model and the number of sensory cells per unit area counted under the microscope. The total number of sensory cells in the nasal sac so arrived at was 800,000.

In spite of the differences mentioned, the two types of cells of the olfactory epithelium are not nearly as distinct in *Petromyzon* as in *Myxine* and other vertebrates. In most other respects the structure of the olfactory mucosa in *Petromyzon* does not differ greatly from that in fishes (8).

Considerable attention has been given in the literature to the ontogenetic relationships between the olfactory epithelium on the faces of the lamellae and the indifferent epithelium found at the free central edges and at the bases

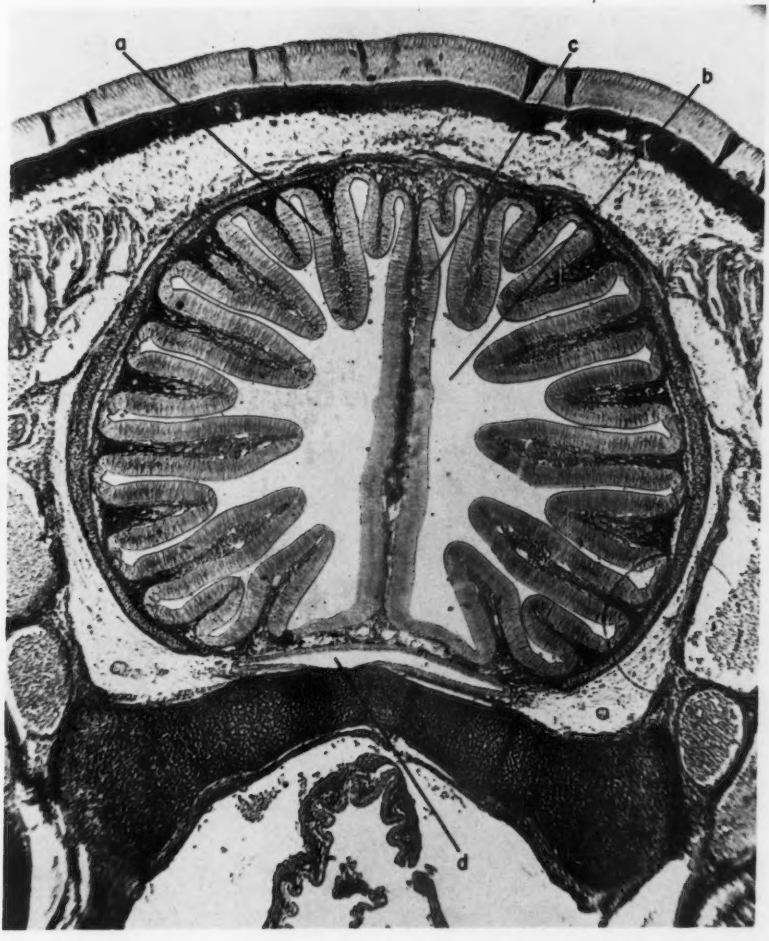

FIG. 8. Cross-section through the olfactory sac of *P. marinus* (13 cm). *a*, lamellum; *b*, lumen; *c*, median lamellum or "septum"; *d*, nasopharyngeal pouch.

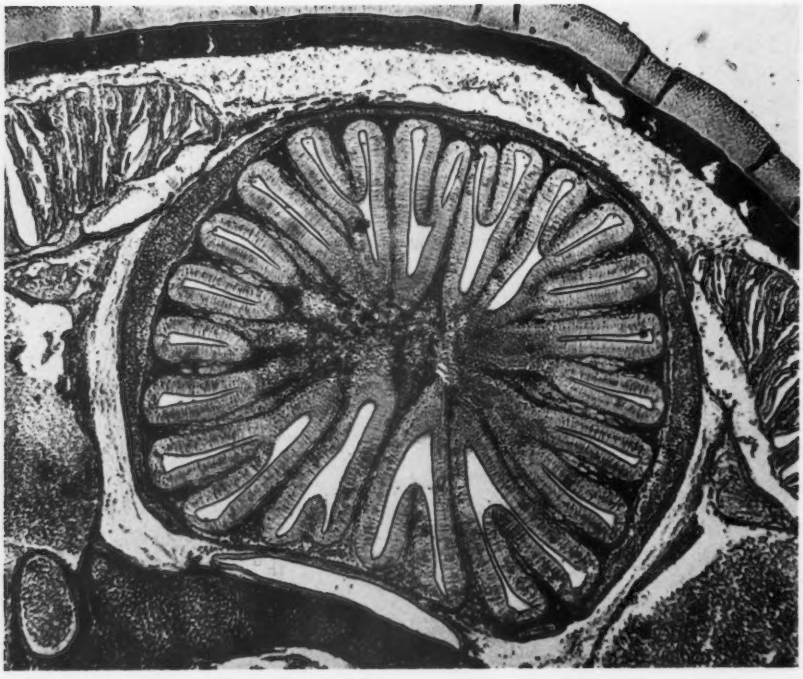

FIG. 9. Cross-section through the caudal extremity of the olfactory sac of *P. marinus* (13 cm), caudal of section shown in Fig. 8.

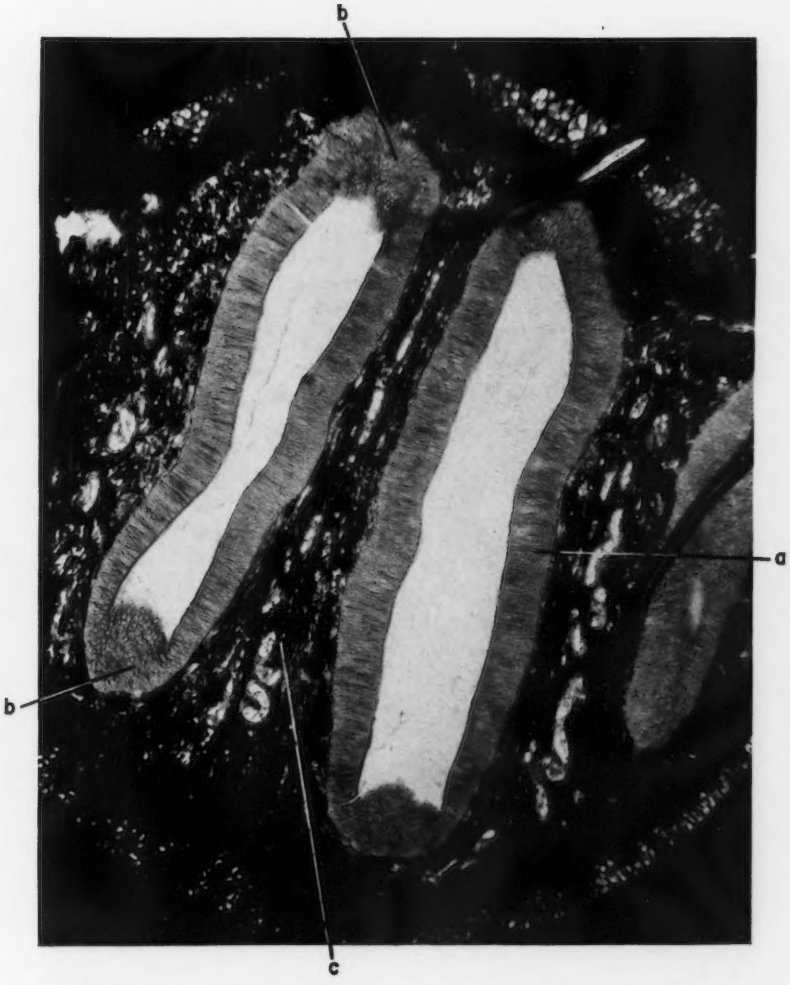

FIG. 10. Olfactory epithelium of *P. marinus*. *a*, sensory epithelium; *b*, indifferent epithelium; *c*, connective tissue with pigment cells.

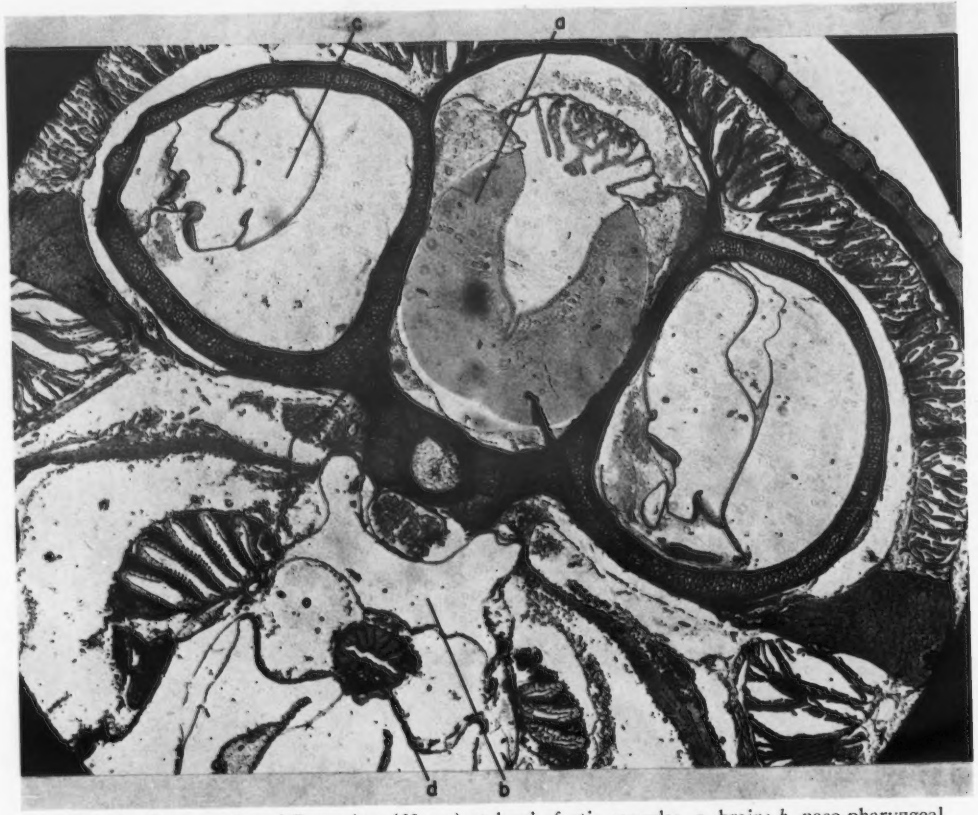

FIG. 11. Cross-section of *P. marinus* (30 cm) at level of otic capsules. *a*, brain; *b*, naso-pharyngeal pouch; *c*, otic capsules; *d*, digestive tract.

of the lamellae (37, 38, 46, 27). Peter (46) showed that the indifferent epithelium is formed from the sensory epithelium which at first exclusively lines the developing nasal sac. Folds in the sensory epithelium are formed before the nature of the tissue changes at their free edges. Only at a later stage the epithelium at the edges and at the bases flattens out and assumes the characteristics of indifferent non-sensory tissue which is much like that which lines the nasal tube (39).

The lamellae in *Petromyzon* arise radially from all the walls of the nasal sac and converge towards the center. Although its presence has been denied in adult *Petromyzon* (14, 50) a median lamella divides the sac into two halves referred to earlier (Fig. 8). Connective tissue forms the axis of each lamella; it is characterized in *Petromyzon* by abundant pigment cells whose black color can be seen through the mucosa (Fig. 10, *c*). The pigmentation is absent in the nasal epithelium of the ammocoete and develops during metamorphosis (see below). The connective tissue forms peripherally an envelope around the sac separating the olfactory epithelium from the cartilaginous tissue which surrounds the sac (14).

In the ammocoete the shape of the nasal sac is that of an oblong hemisphere which is connected with the nasal tube by a median slit which runs ventrally along the whole length of the sac. In this stage the sac is surrounded only by a layer of connective tissue which lacks cartilaginous reinforcement but for a small U-shaped cartilage covering the sac dorsolaterally (31, 55, 28). This cartilage is the forerunner of the nasal capsule in the adult.

In the ammocoete stage the structure of the olfactory epithelium of the nasal sac is similar to that in the adult. It is, however, flat but for a single longitudinal fold originating from a median dorsal line. This fold reaches the floor of the sac only in its posterior portion; farther orad it stays short of the floor and still farther orad it barely indicates the median symmetry which is distinct in the caudal part of the sac. The curvature of the oblong hemisphere brings about a gradual narrowing of the lumina of the two chambers posteriorly where finally small tubular spaces are separated from the lumina by ingrowths from the walls. Further reference to this will be made below (see accessory organ).

The radial lamellae of the adult arise during metamorphosis as longitudinal folds of the olfactory epithelium. The first folds to be formed are horizontal, one in each chamber, in the direction of its long axis so that now four chambers are present. Subsequent development of radially oriented folds further subdivides the lumina of the chambers until, in *P. marinus*, a total of 25 secondary chambers are formed; in the animal used for the wax model there are 12 on the left, 13 on the right side. At the end of metamorphosis all lamellae have attained approximately the same length but for the median one which, by coalescing with the floor of the sac (see above) forms a septum which divides the sac in two halves but for its oral extremity (Fig. 8). This development of the median fold in cyclostomes has received much attention in the past since it sheds light on the nature of the original symmetry of the nasal apparatus in these animals (32, 28, 50).

The Accessory Organ

Posterior to the folds of the olfactory epithelium an aggregation of vesicles is found which is surrounded by pigment cells (Fig. 11). The vesicles are more or less circular in cross-section and imbedded in a dense mass of blood corpuscles which are enclosed in large sinuses of irregular shape. The vesicles aggregate in groups each of which is in contact with the base of a fold of the olfactory epithelium (6). Rami from the olfactory nerve enter the vesicular aggregates which extend laterally as far as the lateral walls of the nasal sac. According to de Beer (6) the vesicles are not in open connection with any other part of the nasal organ. The position of the groups of vesicles close to the lamellae results from the formation of the vesicles by proliferation of cells of the involutions of the epithelium which form the lamellae. There are, however, no open connections between the vesicles and the lamellae. The absence or presence of such connections gains importance with respect to the possible functions of this peculiar organ in *Petromyzon*. The vesicles are lined by large cuboidal tapered cells whose narrow extremity contains a well-defined nucleus and points away from the lumen of the vesicles. Cilia cover the free surface of these cells (6). Several authors (50, 30, 3, 39) have attributed glandular function to this vesicular organ referred to in the literature as accessory organ or follicular appendages. Some authors found evidence of ducts connecting the organ with the nasal sac (3, 25) but others have denied the existence of such ducts (58, 46, 70, 5). In our own material no ducts could be found in a complete series of 10 μ sections of the organ. The presence of sensory cells in the vesicles and their supply by nerve fibers which seem to be identical with those which lead from the olfactory epithelium suggests that the organ may have an olfactory function (25). How such olfactory function could be useful in the absence of ducts to the nasal sac or even in the presence of microscopically narrow ducts such as described by Ballowitz (2, 25) is difficult to understand. The fact that the vesicles are surrounded by blood corpuscles suggested glandular function to de Beer (6). However, to date, there is no physiological evidence for this or any other function of the accessory organ. There is, of course, the possibility that the organ is not functional in present-day *Petromyzon* and that it represents rudiments of a part of the nasal sac, a view presented by Lubosch (37, 38). Scott (58), who first observed this peculiar organ in *Petromyzon*, believed it to be homologous with Jacobson's organ while Woerdeman (70) considered it a homologue of the pars tuberalis of the hypophysis of higher vertebrates. Scott's views have been rejected by Peter (46) and Dieulafé (14), Woerdeman's by de Beer (5) and Matthes (39). Green (23), re-examining the problem, felt that in the accessory organ three zones can be distinguished which may be comparable to the pars tuberalis, pars distalis, and pars intermedia of other vertebrates. Homology with Bowman's glands in man and other mammals is proposed by Leach (33), who also points out that the organ is best developed in those non-parasitic lampreys which lack a posttransformation growth period.

The developmental origin of the accessory organ is found in the coalescence of the posterior and anterior edges of the developing lamellae. Caudad this

coalescence is also with the median fold. The lumina at this extremity become blind sacs or vesicles which assume the histological characteristics described above. These vesicles are present in the larva but become much more numerous in the adult (28).

The Nasopharyngeal Pouch

Reference was made earlier in this paper to the posterior extension of the nasal tube which develops during metamorphosis and which forms a large pouch (Fig. 1, *g*) in the adult. In Petromyzontidae this pouch ends blindly in the vicinity of the second branchial opening. It does not communicate with the gut as in Myxinoidea (61). The development of this unusual organ is referred to on page 213. Various workers have studied the origin and homology of the pouch which has received various names in the literature: nasal caecum (13), posterior nasal tube (12), nasohypophyseal organ (64), nasopharyngeal pouch (33). The variety in this nomenclature points to lack of agreement among these authors on the homology and origin of the organ. Leach (33) has reviewed the literature and considers the pouch as a precursor of the nasopharyngeal passageway of the higher vertebrates. He denies that there is either a morphological or histological connection between the pouch and the hypophysis. According to that author the lumen of the pouch during its development was mistaken by various observers (5, 15, 30) for a hypophyseal cavity. Goette (22) also believed the pouch to belong to the hypophysis. Tilney (64) rejected this relationship.

The pouch is only little developed in the ammocoete. It runs closely below the forebrain and midbrain and ends near the anterior tip of the chord below the regio infundibulis. There is a very small lumen cephalad since the walls are here closely pressed together, but caudad the walls separate and form a small broad pouch. During metamorphosis the pouch grows caudad and ventrad since it is prevented from growth in horizontal direction by the presence of the chorda dorsalis. Just in front of the chorda the tube perforates the still membranous base of the brain capsule and continues its growth caudad between the chorda and the gut surrounded by connective tissue. The walls of the tube now separate further, leaving a larger lumen. At its posterior end the tube finally widens into a large pouch by lateral expansion of its walls. A dorsoventral compression in its median plane is brought about by the solid cord of cells which forms the non-functional gut in the transforming animal (28). In *P. marinus* this compression persists in the adult, giving the pouch its peculiar shape.

The blind caudal part of the pouch has soft flexible walls which are subject to the rhythmic contractions and dilations of the branchial chamber. As early as the first half of the 19th century the function of the pouch in cyclostomes was inferred from its position by Joh. Müller (41, 42, 43, 44), who believed that the flexible pouch aspired and expelled water through the nasal tube and nostril as a result of the rhythmic contractions of respiratory muscles in the branchial basket orad of the second internal gill opening (3). A still earlier author (51) reported that the current of water in and out of the

nasal tube resulted from rhythmic contractions of the nasal sac whose capsule would be surrounded by special musculature for this purpose. However, the presence of such muscles was denied by Schneider (55), and quoted by Kaensche (28).

The function of the pouch as a water aspirator was confirmed by Dawson (13) and there can be no doubt about this interpretation. Water is aspired and expelled in synchrony with respiratory movements of the gill pouches which were studied by Dawson (13), Tretjakoff (66), Balabai (1), and Roberts (54) in *L. fluviatilis*. Through the work of the latter author the mechanism of respiratory movements in this lamprey has become better known. Expiration is brought about by a sharp contraction of the branchial constrictor muscles which run an almost semicircular course from the parachordal sheath to the ventral commissure at right angles to the long axis of the gill sac. The contraction of these muscles causes the body wall to move inwards thereby flexing the cartilaginous bars of the basket in several places. Roberts believes that the rapid return of the body wall at the end of expiration is caused by the contraction of the diagonal muscles described by him and not by the elastic recoil of the cartilaginous gill bars. However, direct observation of the sequence of contraction by these muscles does not support this hypothesis on the function of the diagonal muscles.

The water is expelled forcibly from the gill sac during expiration; the moment the expiratory muscles relax a sharp fall in pressure in the gill sac occurs so that water rushes in from the outside. The contractions of the body wall are directly transmitted to the nasopharyngeal pouch causing it to aspire and expel water through the nasal tube and nostril. The aspired water is forced into the nasal sac and into the lumina of the secondary chambers by the action of the nasal valve, as described earlier. Measurements based on our wax model gave a volume proportion of (pouch/nasal sac) = ($14\ mm^3/7\ mm^3$) in a 30-cm long lamprey.

The resulting pulsating current over the olfactory epithelium of the nasal sac is similar to that found in some of the higher fishes. In *Gasterosteus aculeatus*, siluroids, *Clupea*, *Blennius*, *Zeus*, and others a pulsating current is produced by the rhythmic contractions of accessory nasal sacs (60, 7, 67, 47, 48). There is no evidence in adult *Petromyzon* that the current of water is further promoted by ciliar movements as described for *Pleuronectus platessa* and other fishes (67).

The Origin of Monorhiny in Cyclostomes

It is now generally believed that monorhiny in cyclostomes is secondary in origin and that it has developed from earlier amphirhiny. In *P. marinus* the presence of a pair of olfactory nerves support this view (11, 20, 58). Bütschli (9) has, however, pointed out that the olfactory nerve consists of a complex of nerve elements which have their origin in the sensory cells of the olfactory epithelium. According to Leach (33) the single nasopharyngeal pouch in the non-parasitic lamprey *Ichthyomyzon fossor* may also have arisen from a paired organ. The development of monorhiny in cyclostomes may have resulted from the parasitic life of most of these forms (39).

Monorhiny must have arisen at a very early stage of evolutionary development since it is already present in the fossil cephalospids described by Stensiö (62, 63). Some authors feel, however, that amphirhiny is secondary in nature and that it arose through a division of the original single organ into two parts (19, 24). Kuppfer's view (30) that three placodes participate in the formation of the olfactory organ and that amphirhiny arises from the lateral placodes while the central placode gives origin to the organ in the cyclostomes is now only of historical interest (65).

Innervation

Cords (11) studied the innervation of the olfactory organ in *P. marinus*. A strong nerve leaves the oral-median periphery of the bulbus olfactorius and runs directly to the nasal capsule. This nerve is very short since the nasal sac lies close to the forebrain from which it is separated by the cartilaginous capsule of the sac. Cords objects to the term "lobus olfactorius" in *P. marinus* since the internal structure of the frontal part of the telencephalon corresponds better to that of the bulbus. The olfactory tract consists of numerous bundles which are surrounded by a strong sheath of dura. The tract enters the nasal capsule through the foramen olfactorii from where the nerve fila distribute into the olfactory epithelium and into the follicles of the accessory organ (Fig. 11).

Olfactory Function

The function of the nasal organ of *P. marinus* is now being studied specifically with respect to the nature of the chemical substances emanated by prey which stimulate the olfactory epithelium of this species. The results of this study will be presented in a separate paper. However, the importance of olfaction to the adult of the species is strongly indicated by the spatial extension and the structural complexity of the nasal organ. This development, which takes place primarily during metamorphosis, and the great simplicity of the organ in the ammocoete, clearly point to a relationship between the nature of the procurement of food in the larva (organic matter in the sediment of streams) and in the adult (parasitic on fish) and the development of the olfactory apparatus. Both the organ and the bulbus olfactorius in the adult are the most conspicuous organs. It is of interest to notice that the size of the olfactory capsule is considerably greater than that of the brain and that the bulbus olfactorius is the most conspicuous part of the brain in *Petromyzon marinus*. On these grounds alone it must, therefore, be assumed that olfaction plays an important rôle in the life of the adult while it may be without importance or even absent in the ammocoete.

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NOTES

STICTYLUS HASTATUS (KHAN, 1957) N. COMB., AND STICTYLUS UNGULACAUDUS (KHAN, 1957) N. COMB. (NEMATODA:NEOTYLENCHIDAE)

M. A. KHAN[1]

Dufour (2) described the genus *Sphaerularia* with *S. bombi* as the type and only species. Later Filipjev (3) established the subfamily Sphaerulariinae to include the genus *Sphaerularia* and all other nematodes living in the body cavity of the insects. Chitwood and Chitwood (1) modified this classification and retained in Sphaerulariinae the two genera *Sphaerularia* and *Tripius*, both of which showed eversion of uterus as a common and normal feature. They placed this subfamily in the family Allantonematidae Chitwood and Chitwood, 1937, within the superfamily Tylenchoidea Chitwood and Chitwood, 1937. I followed this system of classification in describing two new species, *Sphaerularia hastata* (4) and *S. ungulacauda* (5), but the description of *Sphaerularia* had to be emended to enable me to do this (5). Furthermore, some nomenclatorial and systematic changes were anticipated at that time for this group of nematodes when it was stated that "It is not proposed at this stage to divide this genus into two subgenera or genera, but further study of the group may reveal other forms and features so that some changes may become desirable".

The German publications by Ruehm (6) and Wachek (8) were not available to me at the time these two nematodes were described, and Dr. A. D. Baker has since brought the question of the present systematic position of these species to my attention. Wachek (8) transferred three genera, *Sphaerularia* Dufour, 1837, *Tripius* (Leuckart, 1887), and *Scatonema* Bovien, 1932, from Tylenchoidea to Aphelenchoidea, but did not name any families under these superfamilies pending further study. Later Ruehm (6) grouped these genera under Sphaerulariidae (Filipjev, 1934) Skarbilovich, 1947, within Aphelenchoidea. In another part of his paper Ruehm synonymized the genus *Sphaerulariopsis* Wachek, 1955 (8) (Tylenchoidea), with the genus *Stictylus* Thorne, 1941 (7), and thus added eversion of uterus in females as an acceptable feature in *Stictylus*.

Due to the transfer of the generic name *Sphaerularia* to Aphelenchoidea, and with the addition of eversion of uterus in females as a feature in *Stictylus*, a new classification has to be adopted for *Sphaerularia hastata* and *S. ungulacauda*, which, as originally described, had tylenchoid morphological features. In tylenchoids the duct of the dorsal esophageal gland opens into the lumen of the esophagus near the base of the stylet. This duct can best be seen

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in living nematodes and was not distinctly visible in the preserved specimens available to me. However, these two species of nematodes had other tylenchoid characters and features that conform with *Stictylus*, such as the shape of the spicules, caudal bursa covering the entire tail, asymmetrical basal knobs of spear, somewhat irregular shape of the esophagus which is without fibrillations and valve in the bulb, dense texture of the body, and blunt and rounded tail in immature females. In view of the foregoing these two species are now designated as *Stictylus hastatus* (Khan, 1957) n. comb., and *Stictylus ungulacaudus* (Khan, 1957) n. comb., under subfamily Paurodontinae Thorne, 1941 (7), and family Neotylenchidae Thorne, 1949.

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Received October 21, 1959.
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PARASITIC UPTAKE OF RADIOSULPHATE BY TREMATODES[1]

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A frog (*Rana pipiens*), utilized recently for autoradiographic studies of the distribution of mucopolysaccharides in vertebrate organisms, was found to be host of numerous trematodes. This unexpected situation made possible the present observations.

This frog, an adult female of 62 g, had been injected under the dorsal skin with a dose of 1.5 μc $S^{35}O_4$/g wt. in 1 ml weak HCl. It had been kept at 22° C

[1]With gratitude to Professor T. W. M. Cameron for advice and to the Medical Division of the National Research Council for a grant-in-aid.

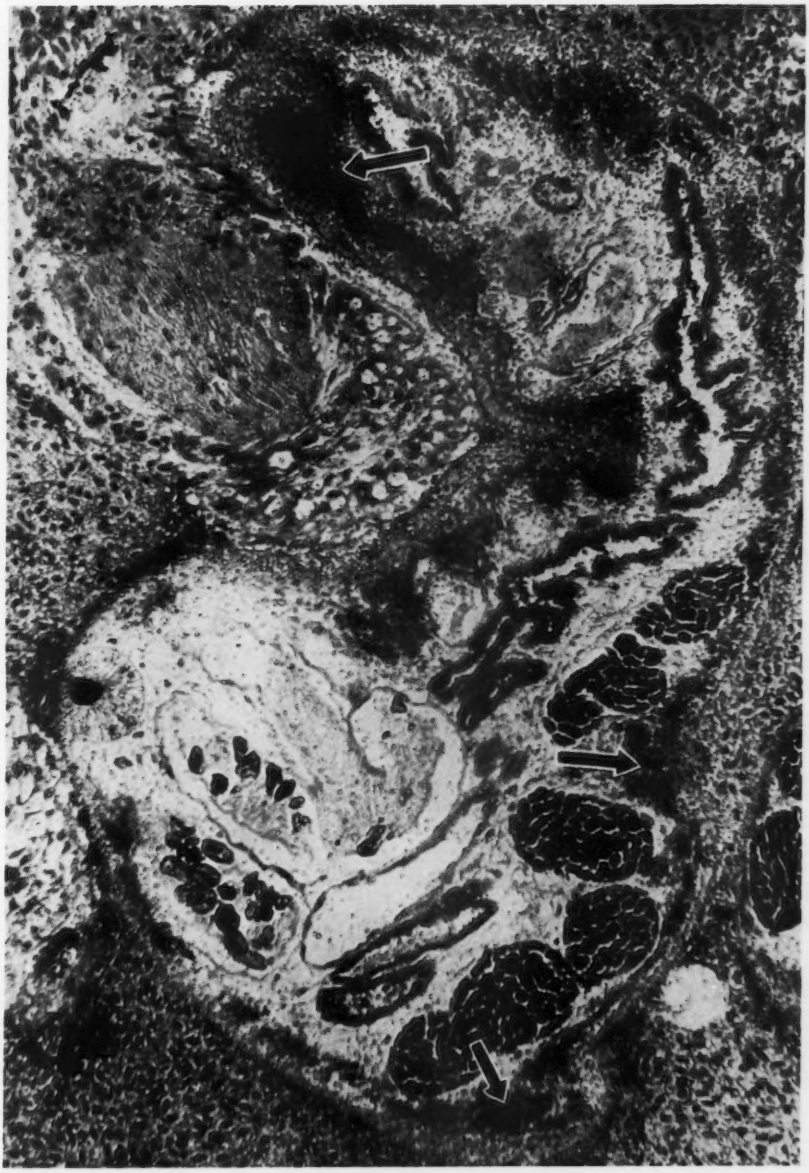

FIG. 1. Sections of a trematode inside the respiratory channels of the frog's lung. Integrated stained autoradiograph, ×214. The arrows point to the autoradiographic record.

for 2 hours then decapitated. Its tissues had been fixed in acetic–formaldehyde–ethanol. Integrated autoradiographs (1, 2) of 10-μ sections had been prepared and exposed for 28 weeks (roughly two half-lives of S^{35}).

The worms were discovered in the lung when the processed autoradiographs were examined under the microscope. These digenetic trematodes, of the genus *Haematoloechus* or *Haplometra* apparently, were found to inhabit the air sacs and bronchi (Fig. 1). The blood adjacent to some of these parasites was most likely aspirated into the lung at the time of decapitation.

The bronchial cartilage of the frog's lung, and the cartilage and fibrous tissue of the heart's "skeleton" on the same slide, revealed practically no uptake of radiosulphate. The mucous cells of the bronchi recorded a small quantity of radioactive material in the distal portion of the cells. It was rather surprising to observe, however, that the worms were intensely radioactive. The tracer was definitely localized under the hypodermis (Fig. 1) at the level of the peripheral musculature (3).

The histochemical stains for acid mucopolysaccharides, Toluidine blue, Hale's iron adsorption technique (4), and Alcian blue (5), were all negative at the level of the trematodes' autoradiographic record. All three reactions were positive at various degrees over the frog's cartilage and dense connective tissue. The radioactive hypodermic band in the worms was positive with the periodic acid-Schiff test for neutral polysaccharides (6). However, other non-radioactive parts of the parasites such as the testes were also strongly P.A.S. positive.

Consequently, the nature of the sulphated substance in the trematodes remains unknown. On the other hand, the intense reaction recorded 2 hours after labelling the host subcutaneously revealed rapid transit of the radiosulphate to the parasite and availability of that substance within the respiratory channels. It also brought out that the metabolism of the trematodes is more intense than that of the frog.

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RECEIVED OCTOBER 13, 1959.
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SOME OBSERVATIONS ON THE DISTRIBUTION AND pH DEPENDENCE OF THE INTESTINAL ALKALINE PHOSPHATASE OF STEELHEAD TROUT (SALMO GAIRDNERII GAIRDNERII)[1]

A. PRAKASH

Histochemical localization of alkaline phosphatase in various animals using Gomori's cobalt sulphide technique has repeatedly shown the presence of this enzyme in the brush border of the intestinal epithelium. Although opinions are divided (2, 4) on the presence of alkaline phosphatase in the nuclei, recent histochemical and biochemical data seem to support the presence of intrinsic phosphatase in this location (1, 2, 3, 5).

In the histochemical localization of alkaline phosphatase in the digestive tract of the steelhead trout using Gormori's method, it was found that in addition to the brush border, alkaline phosphatase activity was present in the nuclei of the columnar epithelial cells of the mucosa of intestine and pyloric caeca and in the lamina propria of the villi. The reaction in the latter was particularly intense in the endothelial cells of the blood capillaries, reticular connective tissue, and lymphatics. At the base of villi, fibroblasts, lymphocytes, and granule cells showed intense reaction. The pattern of alkaline phosphatase distribution was similar in both the pyloric caeca and the intestine. Reaction in the brush border, nuclei, and lamina propria raised some doubts as to whether we are dealing with the same enzyme in all these regions or the phenomenon of 'enzyme plurality' was present.

In order to examine the above in more detail, 5 microns thick sections of pyloric caeca, intestine, and kidney (control) from 12 steelhead trout belonging to same stock were incubated at different pH levels. Incubation time in each case never exceeded 60 minutes. The effect of the pH of the incubation mixture on the activity of the alkaline phosphatase was observed by varying the pH both by using Michaelis's Veronal acetate – hydrochloric acid buffer (pH range 7–9.2) and by adding $M/10$ hydrochloric acid to the incubation mixture. In each case pH was rechecked by Photovolt model 85 electronic pH meter.

From the results presented in Table I and Figs. 1–4 it is clear that the alkaline phosphatase activity in brush border persists over the range from pH 9.4 to pH 8.2, whereas the reaction in lamina propria persists over a smaller range from pH 9.4 to pH 8.7. Nuclear phosphatase has a much more clearly defined pH optimum at pH 8.6–8.7 with activity persisting over the range from pH 9.2 to 8.5.

The relatively greater sensitivity of nuclear phosphatase activity to pH changes as compared with that in the brush border and the lamina propria does not necessarily mean that the enzymes in these sites are different. The establishment of enzyme plurality needs a careful and critical study of the

[1]Work supported by a grant from the National Research Council of Canada.

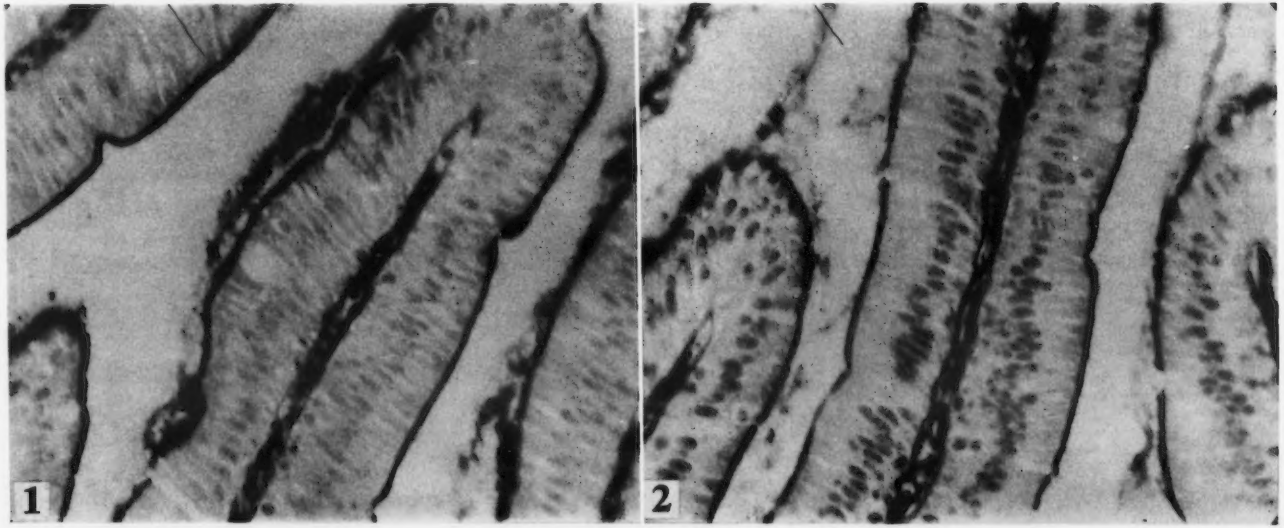

FIG. 1. Incubation at pH 9.4. Brush border and lamina propria show complete reaction. No reaction in the nuclei. ×562

FIG. 2. Incubation at pH 9.2. Complete reaction in brush border and lamina propria, partial reaction visible in nuclei. ×562

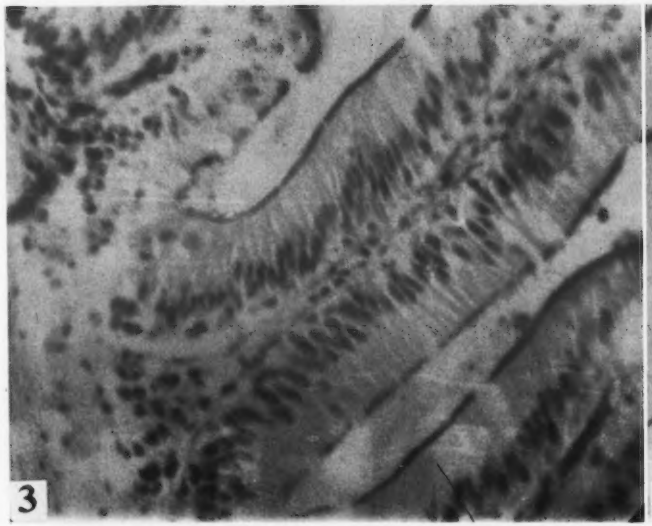

FIG. 3. Incubation at pH 8.6. Complete reaction seen in nuclei, partial reaction in brush border, and no reaction in lamina. ×562

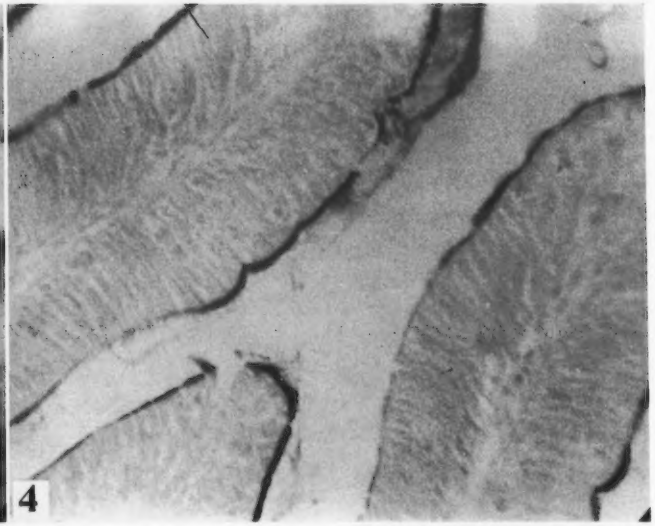

FIG. 4. Incubation at pH 8.2. No reaction visible in nuclei or lamina propria. Only brush border shows partial reaction especially near the tip of villus. ×562

TABLE I

Effect of pH on alkaline phosphatase activity in the various regions of intestine and pyloric caeca

pH	Brush border	Lamina propria	Nuclei
9.4*	++	++	——
9.2*	++	++	+−
8.9	++	+−	+−
8.7	++	+−	++
8.6*	+−	—	++
8.5	+−	—	+−
8.4	+−	—	—
8.3	+−	—	—
8.2*	+−	—	—
8.1	—	—	—
8.0	—	—	—

NOTE: ++, complete reaction; +−, partial reaction; —, no reaction.
*See Figs. 1–4.

kinetics of alkaline phosphatase by histochemical and biochemical methods; nevertheless, the above observations are significant and point to the difference in behavior of the enzyme and do warrant a caution in interpreting histochemical localization of sites of alkaline phosphatase activity.

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RECEIVED SEPTEMBER 28, 1959.
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Canadian Journal of Zoology

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